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- (71) Applicant (for all designated States except US): **GLAXO GROUP LIMITED** [GB/GB]; Glaxo Wellcome House, Berkeley Avenue, Greenford, Middlesex UB6 ONN (GB).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **CWIRLA, Steven, E.** [US/US]; 111 Hedge Road, Menlo Park, CA 94025 (US). **BALU, Palani** [US/US]; 21856 Dolores Avenue, Cupertino, CA 95014 (US). **DUFFIN, David, J.** [US/US]; 1920 Sprunt Avenue, Durham, NC 27705 (US). **PIPLANI, Sunila** [US/US]; 124 Promethean Way, Mountain View, CA 94043 (US). **MCEOWEN-MERRILL, Barbara** [US/US]; 7 Swallows Ridge Court, Durham, NC 27713 (US). **SCHATZ, Peter, J.** [US/US]; 10949 Sycamore Drive, Cupertino, CA 95014 (US).
- (74) Agents: **REED, Dianne, E. et al.**; Reed & Associates, 800 Menlo Park, Suite 210, Menlo Park, CA 94025 (US).
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**WO 02/07676 A2**

(54) Title: COMPOUNDS HAVING AFFINITY FOR THE GRANULOCYTE-COLONY STIMULATING FACTOR RECEPTOR (G-CSFR)

(57) Abstract: Novel compounds are provided that bind to G-CSFR. The novel compounds have a peptide chain approximately 6 to 40 amino acids in length that binds to G-CSFR. The compounds are useful as probes for affinity screening. In addition, the compounds have demonstrated agonist or antagonist activity for the G-CSFR, and are therefore useful in treatment of diseases including patients who suffer from a low white blood cell titer. Pharmaceutical compositions and methods of use are provided as well.

**COMPOUNDS HAVING AFFINITY FOR THE GRANULOCYTE-COLONY  
STIMULATING FACTOR RECEPTOR (G-CSFR)**

5

**TECHNICAL FIELD**

The present invention relates generally to novel compounds that have affinity for the granulocyte-colony stimulating factor receptor (G-CSFR). More particularly, the invention relates to such compounds which act as G-CSF mimetics by activating or inactivating the G-CSFR, or by affecting ligand binding to G-CSFR. The invention additionally relates to methods of using the novel compounds and pharmaceutical compositions containing a compound of the invention as the active agent. The invention has application in the fields of biochemistry and medicinal chemistry and particularly provides G-CSF mimetics for use in the treatment of human disease.

15

**BACKGROUND ART**

Granulocyte-colony stimulating factor (G-CSF) is a hematopoietic growth factor that specifically stimulates proliferation and differentiation of cells of the neutrophilic lineage.

G-CSF is a cytokine that binds to and activates the granulocyte-colony stimulating factor receptor (G-CSFR). G-CSFR is expressed on the surface of mature neutrophils and cells committed to the neutrophilic lineage, with receptor density varying from 190 to more than 1400 sites per cell. The receptor is a member of the cytokine receptor superfamily; it contains a cytokine receptor-homologous domain responsible for G-CSF binding, an immunoglobulin-like domain, three fibronectin type III domains, a transmembrane region, and an intracellular domain. The observed affinity of G-CSF for its receptor is about 100 pM.

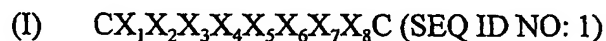
The complete G-CSF protein has become an important therapeutic agent in clinical indications involving depressed neutrophil counts. Such indications include chemotherapy-induced neutropenia, AIDS and community acquired pneumonia.

Furthermore, G-CSF antagonists may be useful in the treatment of some diseases caused by an inappropriate or undesirable activation of G-CSFR.

There remains a need, however, for compounds that bind specifically to G-CSFR, both for studies of the important biological activities mediated by the receptor and for treatment of diseases, disorders and conditions that would benefit from activating or inactivating G-CSFR. The present invention provides such compounds, and also provides pharmaceutical compositions and methods for using the compounds as therapeutic agents.

### **DISCLOSURE OF THE INVENTION**

In one embodiment, the invention provides compounds comprising a peptide chain that binds to G-CSFR. In one aspect, the peptide chain is approximately 10 to 40 amino acids in length and contains a sequence of amino acids of formula (I)



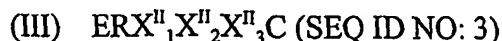
wherein each amino acid is indicated by standard one-letter abbreviation, and wherein  $X_1$  is A, N, S, F, D, G, L, T, E, V, P, Q, H, M or K;  $X_2$  is M, G, R, H, D, I, V, A, S, E, N, F, Y, P, C, W or T;  $X_3$  is E, V, W, F, M, A, N, S, L, T, Y, G or P;  $X_4$  is V, I, G, Q, W, M, T, Y, L, P, D, C, E or A;  $X_5$  is M, E, W, L, P, N, I, T, V, F, Y, Q, S, R, W, G, H or D;  $X_6$  is H, A, W, Y, V, F, Q, M, N, E, S, D, P or G;  $X_7$  is M, F, Y, V, N, L, H, D, S, W, G, Q, C or T; and  $X_8$  is C, Y, R, I, K, W, L, E, M, H, A, T, F, D, P, G or Q.

In another aspect, the peptide chain is approximately 9 to 40 amino acids in length and contains a sequence of amino acids of formula (II)



wherein each amino acid is indicated by the standard one-letter abbreviation, and wherein  $X^I_1$  is S, Q, R, L or Y;  $X^I_2$  is N, S, T, A or D;  $X^I_3$  is E, D or N; and  $X^I_4$  is L, V, T, P or H.

In another aspect, the peptide chain is 6 to 40 amino acids in length and contains a sequence of amino acids of formula (III)



wherein each amino acid is indicated by standard one-letter abbreviation, and wherein  $X^{II}_1$  is D, L, S, G, E, A, K or Y;  $X^{II}_2$  is W, Y, F, L or V; and  $X^{II}_3$  is F, G, M or L.

In still another aspect, the peptide chain is approximately 9 to 40 amino acids in length and contains a sequence of amino acids of formula (IV)

(IV)  $X^{\text{III}}_1\text{MVYX}^{\text{III}}_2\text{X}^{\text{III}}_3\text{PX}^{\text{III}}_4\text{W}$  (SEQ ID NO: 4)

wherein each amino acid is indicated by standard one-letter abbreviation, and wherein  $X^{\text{III}}_1$  is D or E;  $X^{\text{III}}_2$  is A or T;  $X^{\text{III}}_3$  is Y or V; and  $X^{\text{III}}_4$  is P or Y.

In an additional aspect, the invention provides compounds comprising a peptide chain approximately 12 to 40 amino acids in length and contains a sequence of amino acids of formula (V)

(V)  $\text{CX}^{\text{IV}}_1\text{X}^{\text{IV}}_2\text{X}^{\text{IV}}_3\text{X}^{\text{IV}}_4\text{X}^{\text{IV}}_5\text{X}^{\text{IV}}_6\text{X}^{\text{IV}}_7\text{X}^{\text{IV}}_8\text{X}^{\text{IV}}_9\text{X}^{\text{IV}}_{10}\text{C}$  (SEQ ID NO: 5)

wherein each amino acid is indicated by standard one-letter abbreviation, and wherein  $X^{\text{IV}}_1$  is E, G, P, N, R, T, W, S, L, H, A, Q or Y;  $X^{\text{IV}}_2$  is S, T, E, A, D, G, W, P, L, N, V, Y, R or M;  $X^{\text{IV}}_3$  is R, Y, V, Q, E, T, L, P, S, K, M, A or W;  $X^{\text{IV}}_4$  is L, M, G, F, W, R, S, V, P, A, D, C or T;  $X^{\text{IV}}_5$  is V, T, A, R, S, L, W, C, I, E, P, H, F, D or Q;  $X^{\text{IV}}_6$  is E, Y, G, T, Q, M, S, N, A or P;  $X^{\text{IV}}_7$  is C, V, D, G, L, W, E, V, I, S, M or A;  $X^{\text{IV}}_8$  is S, Y, A, W, P, V, L, Q, G, K, F, I, E or D;  $X^{\text{IV}}_9$  is R, W, M, D, H, V, G, A, Q, L, S, E or Y;  $X^{\text{IV}}_{10}$  is M, L, I, S, V, P, W, F, T, Y, R, or Q.

In another aspect the peptide chain is approximately 9 to 40 amino acids in length and contains a sequence of amino acids of formula (VI)

(VI)  $\text{X}^{\text{V}}_1\text{X}^{\text{V}}_2\text{X}^{\text{V}}_3\text{X}^{\text{V}}_4\text{X}^{\text{V}}_5\text{CX}^{\text{V}}_7\text{X}^{\text{V}}_8$  (SEQ ID NO: 6)

wherein each amino acid is indicated by standard one-letter abbreviation, and wherein  $X^{\text{V}}_1$  is E, C, Q, V, or Y;  $X^{\text{V}}_2$  is E, A, L, M, S, W, or Q;  $X^{\text{V}}_3$  is K, R or T;  $X^{\text{V}}_4$  is L, A, or V;  $X^{\text{V}}_5$  is R, A, M, H, E, V, L, G, D, Q, or S;  $X^{\text{V}}_6$  is E or V;  $X^{\text{V}}_7$  is A or G;  $X^{\text{V}}_8$  is R, H, G or L.

In a further aspect, the peptide chain is approximately 10 to 40 amino acids in length that binds to G-CSFR and contains a sequence of amino acids of formula (VII)

(VII)  $\text{X}^{\text{VI}}_1\text{X}^{\text{VI}}_2\text{X}^{\text{VI}}_3\text{X}^{\text{VI}}_4\text{X}^{\text{VI}}_5\text{EX}^{\text{VI}}_6\text{X}^{\text{VI}}_7\text{X}^{\text{VI}}_8\text{X}^{\text{VI}}_9$  (SEQ ID NO: 7)

wherein each amino acid is indicated by standard one-letter abbreviation, and wherein  $X^{\text{VI}}_1$  is A, E or G;  $X^{\text{VI}}_2$  is E, H or D;  $X^{\text{VI}}_3$  is R or G;  $X^{\text{VI}}_4$  is K, Y, M, N, Q, R, D, I, S or E;  $X^{\text{VI}}_5$  is A, S or P;  $X^{\text{VI}}_6$  is E, D, T, Q, K or A;  $X^{\text{VI}}_7$  is R, W, K, L, S, A or Q;  $X^{\text{VI}}_8$  is R or E; and  $X^{\text{VI}}_9$  is W, G, or R.

In a final aspect, the invention also provides peptides that, while not necessarily corresponding to one of the above-defined formulas, bind to G-CSFR.

In some contexts, the compounds of the invention are preferably in the form of a dimer. It is also preferred, in some contexts, that the compounds of the invention include a peptide wherein the N-terminus of the peptide is coupled to a polyethylene glycol

molecule. In some contexts, it is preferred that the compounds of the invention include a peptide wherein the N-terminus of the peptide is acetylated. In addition, it is preferred, in some contexts, that the compounds of the invention include a peptide wherein the C-terminus of the peptide is amidated.

5           The invention also provides a pharmaceutical composition that comprises a therapeutically effective amount of a compound of the invention in combination with a pharmaceutically acceptable carrier, as well as a method for treating a patient who would benefit from a G-CSFR modulator, the method comprising administering to the patient a therapeutically effective amount of a compound of the present invention.

10

### **BRIEF DESCRIPTION OF THE FIGURES**

Figures 1-1, 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, and 1-11 provide the sequences of representative peptide chains contained within the compounds of the invention.

15           Figures 2, 3, 4, 5, 6, 7, 8, 9A, 9B, 10A, 10B, and 11 are graphs showing the results of various assays described in Examples.

### **DETAILED DESCRIPTION OF THE INVENTION**

#### **I. DEFINITIONS AND OVERVIEW**

20           It is to be understood that unless otherwise indicated, this invention is not limited to specific peptide sequences, molecular structures, pharmaceutical compositions, or the like, as such may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be  
25           limiting.

          It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a novel compound" in a pharmaceutical composition means that more than one of the novel compounds can be  
30           present in the composition, reference to "a pharmaceutically acceptable carrier" includes combinations of such carriers, and the like.

In this specification and in the claims that follow, reference will be made to a number of terms which shall be defined to have the following meanings:

Amino acid residues in peptides are abbreviated as follows: Phenylalanine is Phe or F; Leucine is Leu or L; Isoleucine is Ile or I; Methionine is Met or M; Valine is Val or V; Serine is Ser or S; Proline is Pro or P; Threonine is Thr or T; Alanine is Ala or A; Tyrosine is Tyr or Y; Histidine is His or H; Glutamine is Gln or Q; Asparagine is Asn or N; Lysine is Lys or K; Aspartic Acid is Asp or D; Glutamic Acid is Glu or E; Cysteine is Cys or C; Tryptophan is Trp or W; Arginine is Arg or R; and Glycine is Gly or G. In addition, "1-Nal" is used to refer to 1-naphthylalanine, the "2-Nal" is used to refer to 2-naphthylalanine.

Stereoisomers (e.g., D-amino acids) of the twenty conventional amino acids, unnatural amino acids such as  $\alpha,\alpha$ -disubstituted amino acids, N-alkyl amino acids, lactic acid, and other unconventional amino acids may also be suitable components for compounds of the present invention. Examples of unconventional amino acids include:  $\beta$ -alanine, 1-naphthylalanine, 2-naphthylalanine, 3-pyridylalanine, 4-hydroxyproline, O-phosphoserine, N-acetyls erine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, nor-leucine, and other similar amino acids and imino acids (e.g., 4-hydroxyproline).

"Peptide" or "polypeptide" refers to a polymer in which the monomers are alpha amino acids joined together through amide bonds. Peptides are two or often more amino acid monomers long. One or more of the peptide chains disclosed herein may appear in the compounds of the present. It is also contemplated that the peptide chains disclosed herein represent only a portion of the overall peptide included in the compound.

The term "dimer" as in a peptide "dimer" refers to a compound in which two peptide chains are linked; generally, although not necessarily, the two peptide chains will be identical and are linked through a linking moiety covalently bound to the carboxyl terminus of each chain.

The term "agonist" is used herein to refer to a ligand that binds to a receptor and activates the receptor.

The term "antagonist" is used herein to refer to a ligand that binds to a receptor without activating the receptor. Antagonists are either competitive antagonists or

noncompetitive antagonists. A "competitive antagonist" blocks the receptor site that is specific for the agonist. A "noncompetitive antagonist" inactivates or otherwise affects the functioning of the receptor by interacting with a site other than the agonist binding site.

5 The term "modulator" as in a "G-CSFR-modulator" refers to a compound that is either an agonist or antagonist of the G-CSFR.

"Pharmaceutically or therapeutically effective dose or amount" refers to a dosage level sufficient to induce a desired biological result. That result can be alleviation of the signs, symptoms, or causes of a disease, or any other desired alteration of a biological system. Preferably, this dose or amount will be sufficient to either at least partially  
10 activate or at least partially inactivate G-CSFR and, thus, alleviate the symptoms associated with an undesired neutrophil count *in vivo*.

An "optimal neutrophil count" refers to a quantity of neutrophils in a patient that is determined by a clinician to be optimal for that patient in light of the patient's disease state, condition, etc.

15 An "undesired neutrophil count" refers to a quantity of neutrophils in a patient that is determined by a clinician to be not optimal for that patient in light of the patient's disease state, condition, etc. Thus, an undesired neutrophil count may be depressed, elevated or even equal to the expected neutrophil count so long as the clinician determines that the actual count is not optimal for the patient. The compounds of the present  
20 invention are intended to, *inter alia*, provide the clinician with compounds that, when administered to a patient, bring that patient's neutrophil count closer to an optimal neutrophil count.

The term "treat" as in "treat a disease" is intended to include any means of treating a disease in a mammal, including (1) preventing the disease, i.e., avoiding any  
25 clinical symptoms of the disease, (2) inhibiting the disease, that is, arresting the development or progression of clinical symptoms, and/or (3) relieving the disease, i.e., causing regression of clinical symptoms.

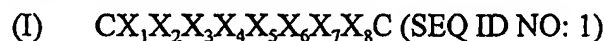
"Optional" or "optionally" means that the subsequently described circumstance may or may not occur, so that the description includes instances where the circumstance  
30 occurs and instances where it does not.

By "pharmaceutically acceptable carrier" is meant a material which is not biologically or otherwise undesirable, i.e., the material may be administered to an individual along with the selected active agent without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained.

## II. THE NOVEL COMPOUNDS

### A. COMPOUNDS OF FORMULA (I):

In a first embodiment, the invention provides compounds comprising a peptide chain that binds to G-CSFR, wherein the compounds comprise a peptide chain approximately 10 to 40 amino acids in length that binds to G-CSFR and contains a sequence of amino acids of formula (I)



wherein each amino acid is indicated by standard one-letter abbreviation, and wherein  $X_1$  is A, N, S, F, D, G, L, T, E, V, P, Q, H, M or K;  $X_2$  is M, G, R, H, D, I, V, A, S, E, N, F, Y, P, C, W or T;  $X_3$  is E, V, W, F, M, A, N, S, L, T, Y, G or P;  $X_4$  is V, I, G, Q, W, M, T, Y, L, P, D, C, E or A;  $X_5$  is M, E, W, L, P, N, I, T, V, F, Y, Q, S, R, W, G, H or D;  $X_6$  is H, A, W, Y, V, F, Q, M, N, E, S, D, P or G;  $X_7$  is M, F, Y, V, N, L, H, D, S, W, G, Q, C or T; and  $X_8$  is C, Y, R, I, K, W, L, E, M, H, A, T, F, D, P, G or Q.

Preferably  $X_1$  is D or P;  $X_2$  is D or P;  $X_3$  is E or W;  $X_4$  is V, I or Y;  $X_5$  is M or L;  $X_6$  is W, Y or F;  $X_7$  is M, Y or D; and  $X_8$  is C or M.

Examples of particularly preferred sequences satisfying formula (I) include, but are not limited to, the following:

CAGEVMHMCC (SEQ ID NO: 8);  
 CNREIEAMCC (SEQ ID NO: 9);  
 CADEVMHFCC (SEQ ID NO: 10);  
 CNREIMWMCC (SEQ ID NO: 11);  
 CSHEVWWYCC (SEQ ID NO: 12);  
 CSREVLYYCC (SEQ ID NO: 13);  
 CFIEGPWVCC (SEQ ID NO: 14);  
 CFVEGNWYCC (SEQ ID NO: 15);



CAAHEVMVNCC (SEQ ID NO: 16);  
CSDEVIFYCC (SEQ ID NO: 17);  
CDREIMWFCC (SEQ ID NO: 18);  
CAHEVMWMCC (SEQ ID NO: 19);  
5 CGSEVTFMCC (SEQ ID NO: 20);  
CLEEIMWLCC (SEQ ID NO: 21);  
CAREVLAMCC (SEQ ID NO: 22);  
CSVEVMQMCC (SEQ ID NO: 23);  
CTNVQLMHYC (SEQ ID NO: 24);  
10 CDVWQLFDRC (SEQ ID NO: 25);  
CSFVQLNSIC (SEQ ID NO: 26);  
CDYWQWFDKC (SEQ ID NO: 27);  
CESFWVELWC (SEQ ID NO: 28);  
CVPWMFYDLC (SEQ ID NO: 29);  
15 CDPWMFYDLC (SEQ ID NO: 30);  
CDPWVLFDEC (SEQ ID NO: 31);  
CDHWTYFDMC (SEQ ID NO: 32);  
CVVWTLYDKC (SEQ ID NO: 33);  
CPDWYQSYMC (SEQ ID NO: 34);  
20 CPDWYSYYMC (SEQ ID NO: 35);  
CPEWYTDVMC (SEQ ID NO: 36);  
CPDWYLDYMC (SEQ ID NO: 37);  
CPEWYLDYMC (SEQ ID NO: 38);  
CPDWYLPYMC (SEQ ID NO: 39);  
25 CPEWYLPYMC (SEQ ID NO: 40);  
CQDWWVELWC (SEQ ID NO: 41);  
CPDWYLPWMC (SEQ ID NO: 42);  
CACMLRVVHC (SEQ ID NO: 43);  
CQRAGYMLAC (SEQ ID NO: 44);  
30 CHANPVWGEC (SEQ ID NO: 45);  
CFWSDWGQTC (SEQ ID NO: 46);

CPHWTSYYMC (SEQ ID NO: 47);  
CETLCGACFC (SEQ ID NO: 48);  
CATTINDTLC (SEQ ID NO: 49);  
CLNYPHPVFC (SEQ ID NO: 50);  
5 CMDGEMAVDC (SEQ ID NO: 51);  
CNMGWMSWPC (SEQ ID NO: 52)  
CETYADWLGC (SEQ ID NO: 53);  
CDPWMFFDMC (SEQ ID NO: 54);  
CDPWIWYDLC (SEQ ID NO: 55);  
10 CDPWIMYDRC (SEQ ID NO: 56);  
CDPWVFFDIC (SEQ ID NO: 57);  
CDPWTYYDLC (SEQ ID NO: 58);  
CDPWIFYDRC (SEQ ID NO: 59);  
CDPWLFYDLC (SEQ ID NO: 60);  
15 CDPWVWYDLC (SEQ ID NO: 61);  
CDPWIFFDRC (SEQ ID NO: 62);  
CDPWMFFDQC (SEQ ID NO: 63);  
CDPWLWYDRC (SEQ ID NO: 64);  
CDVWVWYDQC (SEQ ID NO: 65);  
20 CDPWIYYDLC (SEQ ID NO: 66);  
CVPWTLFDLC (SEQ ID NO: 67);  
CPAWYLEYMC (SEQ ID NO: 68);  
CPDWYLEYMC (SEQ ID NO: 69);  
CKYWQWFDKC (SEQ ID NO: 70); and  
25 CDHWMWYDKC (SEQ ID NO: 71).

Other preferred formula (I) sequences include, but are not limited to the following:

GCNREIEAMCCG (SEQ ID NO: 72);  
GCPEWYTDVMCG (SEQ ID NO: 73);  
30 NWYCMDGEMAVDCEAT (SEQ ID NO: 74);  
WQSCNMGWMSWPCYFV (SEQ ID NO: 75);

HELCETYADWLGCVEW (SEQ ID NO: 76);  
 PCDPWMFFDMCERW (SEQ ID NO: 77);  
 LRGCDPWIWYDLCPAV (SEQ ID NO: 78);  
 GYLCDPWIFYDRCLGF (SEQ ID NO: 79);  
 5. RFACDPWVFFDICGYW (SEQ ID NO: 80);  
 GYWCDPWTTYDLCLTA (SEQ ID NO: 81);  
 MWTCDPWIFYDRCFLN (SEQ ID NO: 82);  
 GSSCDPWLFYDLCLLD (SEQ ID NO: 83);  
 GGGCDPWVWYDLCWCD (SEQ ID NO: 84);  
 10 YTSCDPWIFFDRCMSV (SEQ ID NO: 85);  
 DPYCDPWMFFDQCAYL (SEQ ID NO: 86);  
 REFCDPWLWYDRCL (SEQ ID NO: 87);  
 NTGCDVWVWYDQCFAM (SEQ ID NO: 88);  
 LVFCDPWIYYDLCMDT (SEQ ID NO: 89);  
 15 GCSFVQLNSICG (SEQ ID NO: 90);  
 GCPAWYLEYMCG (SEQ ID NO: 91);  
 GCPDWYLEYMCG (SEQ ID NO: 92);  
 GCKYWQWFDKCG (SEQ ID NO: 93); and  
 GCDHWMWYDKCG (SEQ ID NO: 94).  
 20

#### B. COMPOUNDS OF FORMULA (II):

In another aspect, compounds are provided comprising a peptide chain approximately 9 to 40 amino acids in length that binds to G-CSFR and contains a sequence of amino acids of formula (II)

25 (II)  $X^I_1 X^I_2 X^I_3 S G W V W X^I_4$  (SEQ ID NO: 2)

wherein each amino acid is indicated by the standard one-letter abbreviation, and wherein  $X^I_1$  is S, Q, R, L or Y;  $X^I_2$  is N, S, T, A or D;  $X^I_3$  is E, D or N; and  $X^I_4$  is L, V, T, P or H.

Preferably  $X^I_1$  is S or Q;  $X^I_2$  is S;  $X^I_3$  is N; and  $X^I_4$  is V.

30 Examples of particularly preferred sequences satisfying formula (II) include, but are not limited to, the following:

SNESGWVWL (SEQ ID NO: 95);

QNSNGWVWV (SEQ ID NO: 96);  
RTESGWVWT (SEQ ID NO: 97);  
RANSGWVWV (SEQ ID NO: 98);  
YDNSGWVWH (SEQ ID NO: 99); and  
5 LSDSGWVWVP (SEQ ID NO: 100).

Other preferred formula (II) sequences include, but are not limited to, the following:

EQNSGWVWVGGGGC (SEQ ID NO: 101);  
10 EQNSGWVWV (SEQ ID NO: 102);  
EQNSGWVWVGGGGCKKK (SEQ ID NO: 103);  
EQNSGWVWVGKKKC (SEQ ID NO: 104);  
EQNSGWVWVGKKK (SEQ ID NO: 105);  
KKKEQNSGWVWV (SEQ ID NO: 106);  
15 EQNSGWVWVGKKKSKKK (SEQ ID NO: 107);  
EQNSGWVWVGGCKKK (SEQ ID NO: 108);  
EQNSGWVWVGGGGGGCKKK (SEQ ID NO: 109);  
SNESGWVWLP (SEQ ID NO: 110);  
EQNSGWVWV (SEQ ID NO: 111);  
20 SRTEGWVWT (SEQ ID NO: 112);  
QRANSGWVWV (SEQ ID NO: 113);  
DYDNSGWVWH (SEQ ID NO: 114);  
EQNSGWVWVGKKKK (SEQ ID NO: 115);  
EQNSGWVWVGGGGSKKK (SEQ ID NO: 116);  
25 EQNSGWVWVGGGGS (SEQ ID NO: 117);  
EQNSGWVWVGGGGSEQNSGWVWVGGGGS (SEQ ID NO: 118);  
RYQSFELSDSGWVWVPVARH (SEQ ID NO: 119); and  
EQNSGWVWVGGGGCKKKC (SEQ ID NO: 492).

**C. COMPOUNDS OF FORMULA (III):**

In another aspect, the invention provides compounds comprising a peptide chain approximately 6 to 40 amino acids in length that binds to G-CSFR and contains a sequence of amino acids of formula (III)

5 (III)  $ERX^{\text{II}}_1X^{\text{II}}_2X^{\text{II}}_3C$  (SEQ ID NO: 3)

wherein each amino acid is indicated by standard one-letter abbreviation, and wherein  $X^{\text{II}}_1$  is D, L, S, G, E, A, K or Y;  $X^{\text{II}}_2$  is W, Y, F, L or V; and  $X^{\text{II}}_3$  is F, G, M or L.

Preferably,  $X^{\text{II}}_1$  is D or L;  $X^{\text{II}}_2$  is W; and  $X^{\text{II}}_3$  is F.

10 Examples of particularly preferred sequences satisfying formula (III) include, but are not limited to, the following:

ERDWFC (SEQ ID NO: 120);

ERDWGC (SEQ ID NO: 121);

ERLWFC (SEQ ID NO: 122);

15 ERSYFC (SEQ ID NO: 123);

ERGWFC (SEQ ID NO: 124);

EREWFC (SEQ ID NO: 125);

ERAWFC (SEQ ID NO: 126);

ERLYFC (SEQ ID NO: 127);

20 ERYFMC (SEQ ID NO: 128);

ERLFLC (SEQ ID NO: 129);

ERALMC (SEQ ID NO: 130);

ERDVMC (SEQ ID NO: 131); and

ERKWFC (SEQ ID NO: 132).

25

Particularly preferred compounds are of the formula:

ETWGERDWFC (SEQ ID NO: 133);

ETWGERDWGC (SEQ ID NO: 134);

STAERLWFCG (SEQ ID NO: 135);

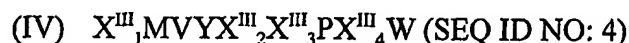
30 YETAERSYFC (SEQ ID NO: 136);

ADNAERGWFC (SEQ ID NO: 137);

QNSEREWFC (SEQ ID NO: 138);  
 STSERAWFCG (SEQ ID NO: 139);  
 ASWSERGWFC (SEQ ID NO: 140);  
 ELSSEREWFC (SEQ ID NO: 141);  
 5 DMQGERGWFC (SEQ ID NO: 142);  
 SSSERAWFCG (SEQ ID NO: 143);  
 GNMRRERYFC (SEQ ID NO: 144);  
 QPNRERYFMC (SEQ ID NO: 145);  
 SVTRERLFLC (SEQ ID NO: 146);  
 10 IPLSERALMCSSWNC (SEQ ID NO: 147);  
 WARSDVMCLSYVC (SEQ ID NO: 148);  
 QNSEREWFCG (SEQ ID NO: 149);  
 QNSEREWFCGGGS (SEQ ID NO: 150);  
 NLEEALAQERLWFCRSGNC (SEQ ID NO: 151); and  
 15 NLESYEMEERKWFCKMFSC (SEQ ID NO: 152).

#### D. COMPOUNDS OF FORMULA (IV):

In another aspect, compounds are provided comprising a peptide chain  
 approximately 9 to 40 amino acids in length that binds to G-CSFR and contains a sequence  
 20 of amino acids of formula (IV):



wherein each amino acid is indicated by standard one-letter abbreviation, and wherein  $X^{\text{III}}_1$   
 is D or E;  $X^{\text{III}}_2$  is A or T;  $X^{\text{III}}_3$  is Y or V; and  $X^{\text{III}}_4$  is P or Y.

Examples of particularly preferred sequences satisfying formula (IV) include,  
 25 but are not limited to, the following:

DMVYAYPPW (SEQ ID NO: 153); and

EMVYTVPYW (SEQ ID NO: 154).

Other preferred formula (IV) sequences include, but are not limited to, the following:

30 DMVYAYPPWS (SEQ ID NO: 155); and

DEMVYTVPYW (SEQ ID NO: 156).

**E. COMPOUNDS OF FORMULA (V):**

In another aspect, compounds are provided comprising a peptide chain approximately 12 to 40 amino acids in length that binds to G-CSFR and contains a sequence of amino acids of formula (V):

5 (V)  $CX^{IV}_1X^{IV}_2X^{IV}_3X^{IV}_4X^{IV}_5X^{IV}_6X^{IV}_7X^{IV}_8X^{IV}_9X^{IV}_{10}C$  (SEQ ID NO: 5)

wherein each amino acid is indicated by standard one-letter abbreviation, and wherein  $X^{IV}_1$  is E, G, P, N, R, T, W, S, L, H, A, Q or Y;  $X^{IV}_2$  is S, T, E, A, D, G, W, P, L, N, V, Y, R or M;  $X^{IV}_3$  is R, Y, V, Q, E, T, L, P, S, K, M, A or W;  $X^{IV}_4$  is L, M, G, F, W, R, S, V, P, A, D, C or T;  $X^{IV}_5$  is V, T, A, R, S, L, W, C, I, E, P, H, F, D or Q;  $X^{IV}_6$  is E, Y, G, T, Q, M, S, N, A or P;  $X^{IV}_7$  is C, V, D, G, L, W, E, V, I, S, M or A;  $X^{IV}_8$  is S, Y, A, W, P, V, L, Q, G, K, F, I, E or D;  $X^{IV}_9$  is R, W, M, D, H, V, G, A, Q, L, S, E or Y;  $X^{IV}_{10}$  is M, L, I, S, V, P, W, F, T, Y, R, or Q.

Preferably  $X^{IV}_1$  is E;  $X^{IV}_2$  is S or A;  $X^{IV}_3$  is R;  $X^{IV}_4$  is L;  $X^{IV}_5$  is V or S;  $X^{IV}_6$  is E;  $X^{IV}_7$  is C;  $X^{IV}_8$  is S;  $X^{IV}_9$  is R; and  $X^{IV}_{10}$  is L.

15 Examples of particularly preferred sequences satisfying formula (V) include, but are not limited to, the following:

CESRLVECSRMC (SEQ ID NO: 157);

CETYMTYVYWLC (SEQ ID NO: 158);

CGERLAECARLC (SEQ ID NO: 159);

20 CESRLRECSMLC (SEQ ID NO: 160);

CEARLSECSRIC (SEQ ID NO: 161);

CPARLLECSRMC (SEQ ID NO: 162);

CESVGVDWWSC (SEQ ID NO: 163);

CEDRLVEGPWVC (SEQ ID NO: 164);

25 CNDQFRTCVDVC (SEQ ID NO: 165);

CRGEWWELYHPC (SEQ ID NO: 166);

CEDTRTGAWWSC (SEQ ID NO: 167);

CTWLSSGELVWC (SEQ ID NO: 168);

CWPPVCEVSGIC (SEQ ID NO: 169);

30 CSLSPIQLQHLC (SEQ ID NO: 170);

CLARLEECSRFC (SEQ ID NO: 171);

CHNSSPMVGVTC (SEQ ID NO: 172);  
CHVSPVQIKALC (SEQ ID NO: 173);  
CAAPATSWFQYC (SEQ ID NO: 174);  
CASKLHECSLRC (SEQ ID NO: 175);  
5 CEPMDSNGIVQC (SEQ ID NO: 176);  
CQYASAADEQRC (SEQ ID NO: 177);  
CEYWDEPSLSWC (SEQ ID NO: 178);  
CERECFQMLERC (SEQ ID NO: 179);  
CGMSTDELDEIC (SEQ ID NO: 180);  
10 CYVSPSTGLYSC (SEQ ID NO: 181);  
CEARLVECSRMC (SEQ ID NO: 182);  
CESRLSECSRMC (SEQ ID NO: 183);  
CELKLQECARRC (SEQ ID NO: 184);  
CELKLQEAARRC (SEQ ID NO: 185); and  
15 CLERLEECSRFC (SEQ ID NO: 186).

Other preferred formula (V) sequences include but are not limited to, the following:

GGCESRLVECSRMC (SEQ ID NO: 187);  
GGCETYMTYVYWLC (SEQ ID NO: 188);  
20 EWLCESVGVDWWSC (SEQ ID NO: 189);  
YHPCEDRLVEGPWVCCRS (SEQ ID NO: 190);  
WLLCNDQFRTCVDVCDNV (SEQ ID NO: 191);  
IAECRGEWWELYHPCCLAA (SEQ ID NO: 192);  
TWYCEDTRTGWAWSCLEL (SEQ ID NO: 193);  
25 QLDCTWLSSGELVWCSDW (SEQ ID NO: 194);  
QFDCTWLSSGELVWCSDW (SEQ ID NO: 195);  
CWPPVCEVSGICS (SEQ ID NO: 196);  
CGCSLSPIQLQHLC (SEQ ID NO: 197);  
CGCHVSPVQIKALC (SEQ ID NO: 198);  
30 GCHVSPVQIKALC (SEQ ID NO: 199);  
GTSCAAPATSWFQYCVLP (SEQ ID NO: 200);



RMDCASKLHECSLRCA YA (SEQ ID NO: 201);  
 GVVCEPMDSNGIVQCSMR (SEQ ID NO: 202);  
 IDVCQYASAADEQRCLRI (SEQ ID NO: 203);  
 NVLCEYWDEPSLSWCLSS (SEQ ID NO: 204);  
 5 CQCERECFQMLERC (SEQ ID NO: 205);  
 FCSCGMSTDELDEICAIW (SEQ ID NO: 206);  
 EEVCYVSPSTGLYSCYDQ (SEQ ID NO: 207);  
 LLDICELKLQECARRCN (SEQ ID NO: 208);  
 GGGLLDICELKLQECARRCN (SEQ ID NO: 209);  
 10 GRTGGGLLDICELKLQECARRCN (SEQ ID NO: 210);  
 LGIEGRTGGGLLDICELKLQECARRCN (SEQ ID NO: 211);  
 LLDICELKLQEAARRCN (SEQ ID NO: 212); and  
 KLLDICELKLQEAARRCN (SEQ ID NO: 213).

15 Particularly preferred formula (V) sequences are selected from the group consisting of:

LLDICELKLQECARRCN (SEQ ID NO: 208);  
 GGGLLDICELKLQECARRCN (SEQ ID NO: 209);  
 GRTGGGLLDICELKLQECARRCN (SEQ ID NO: 210);  
 LGIEGRTGGGLLDICELKLQECARRCN (SEQ ID NO: 211);  
 20 LLDICELKLQEAARRCN (SEQ ID NO: 212); and  
 KLLDICELKLQEAARRCN (SEQ ID NO: 213).

#### F. COMPOUNDS OF FORMULA (VI):

In another aspect, compounds are provided comprising a peptide chain  
 25 approximately 9 to 40 amino acids in length that binds to G-CSFR and contains a sequence  
 of amino acids of formula (VI):

(VI)  $X^V_1X^V_2X^V_3X^V_4X^V_5X^V_6CX^V_7X^V_8$  (SEQ ID NO: 6)

wherein each amino acid is indicated by standard one-letter abbreviation, and wherein  $X^V_1$   
 is E, C, Q, V, or Y;  $X^V_2$  is E, A, L, M, S, W, or Q;  $X^V_3$  is K, R or T;  $X^V_4$  is L, A, or V;  $X^V_5$   
 30 is R, A, M, H, E, V, L, G, D, Q, or S;  $X^V_6$  is E or V;  $X^V_7$  is A or G;  $X^V_8$  is R, H, G or L.

Preferably  $X^V_1$  is E;  $X^V_2$  is A or L;  $X^V_3$  is K or R;  $X^V_4$  is L;  $X^V_6$  is E;  $X^V_7$  is A; and  $X^V_8$  is R.

Examples of particularly preferred sequences satisfying formula (VI) include, but are not limited to, the following:

- 5            EEKLRECAR (SEQ ID NO: 214);
- EARLAECAR (SEQ ID NO: 215);
- CMKLMECAR (SEQ ID NO: 216);
- ELRLRECAH (SEQ ID NO: 217);
- EAKLHECAR (SEQ ID NO: 218);
- 10           ELKLAECAR (SEQ ID NO: 219);
- EARLEECAR (SEQ ID NO: 220);
- EAKLRECAR (SEQ ID NO: 221);
- ELRLAECAR (SEQ ID NO: 222);
- ESRLAECAR (SEQ ID NO: 223);
- 15           EAKLVECAR (SEQ ID NO: 224);
- ESRLRECAR (SEQ ID NO: 225);
- EAKLAECAR (SEQ ID NO: 226);
- QWRLEECAR (SEQ ID NO: 227);
- QLRLEECAR (SEQ ID NO: 228);
- 20           ELRLEECAR (SEQ ID NO: 229);
- EAKLLECAR (SEQ ID NO: 230);
- EARAGVCAG (SEQ ID NO: 231);
- EAKAGVCAG (SEQ ID NO: 232);
- VARLEECAR (SEQ ID NO: 233);
- 25           ELKLDECAR (SEQ ID NO: 234);
- EWRLQECAR (SEQ ID NO: 235);
- EAKLSECAR (SEQ ID NO: 236);
- EARLSECAR (SEQ ID NO: 237);
- ELKLLECAR (SEQ ID NO: 238);
- 30           ELRLQECGR (SEQ ID NO: 239);
- EQKLAECAR (SEQ ID NO: 240);

ELRLQECAR (SEQ ID NO: 241);  
ELKLEECAR (SEQ ID NO: 242);  
ESRLEECAR (SEQ ID NO: 243);  
EATVQECAR (SEQ ID NO: 244);  
5 ELKLQECAR (SEQ ID NO: 245);  
YSRLEECGR (SEQ ID NO: 246);  
ELRLRECAL (SEQ ID NO: 247);  
EARLLECAR (SEQ ID NO: 248);  
ESRLLECAR (SEQ ID NO: 249);  
10 VLKLEECAR (SEQ ID NO: 250);  
ESKLAECAR (SEQ ID NO: 251);  
ESKLRECAR (SEQ ID NO: 252);  
EYKLGECAR (SEQ ID NO: 253);  
ESRLQECAR (SEQ ID NO: 254);  
15 QARLAECAR (SEQ ID NO: 255);  
ELKKQECAR (SEQ ID NO: 256);  
ESRLSECAR (SEQ ID NO: 257);  
EARLEECGR (SEQ ID NO: 258);  
ESRLAECGR (SEQ ID NO: 259);  
20 EWRLEECAR (SEQ ID NO: 260);  
EARLSECGR (SEQ ID NO: 261);  
AARLAECAR (SEQ ID NO: 262);  
EWKLAECAR (SEQ ID NO: 263);  
ESKLEECAR (SEQ ID NO: 264);  
25 DVKLAECAR (SEQ ID NO: 265);  
ELQLEECAR (SEQ ID NO: 266); and  
EYKLASCAR (SEQ ID NO: 267).

Other preferred formula (VI) sequences include but are not limited to, the following:

30 RLSICEEKLRECARGC (SEQ ID NO: 268);  
PLTTCEARLAECARQL (SEQ ID NO: 269);  
LALCMKLMECARRY (SEQ ID NO: 270);

ELVMCELRLRECAHRA (SEQ ID NO: 271);  
PLARCEAKLHECARQL (SEQ ID NO: 272);  
LLSVCELKLAECARSK (SEQ ID NO: 273);  
RLEWCEARLEECARRC (SEQ ID NO: 274);  
5 RLRVVEAKLRECARGR (SEQ ID NO: 275);  
CVAHLELRLAECARQI (SEQ ID NO: 276);  
HLARCESRLAECARQL (SEQ ID NO: 277);  
RLALLEAKLVECARRL (SEQ ID NO: 278);  
DLFSLESRLRECARRV (SEQ ID NO: 279);  
10 AVPVLEAKLAECARRF (SEQ ID NO: 280);  
YLQQLQWRLEECARGM (SEQ ID NO: 281);  
YLELCQLRLEECARQFN (SEQ ID NO: 282);  
ELHICELRLEECARGR (SEQ ID NO: 283);  
RVARCELRLAECARKS (SEQ ID NO: 284);  
15 YLEVLESRLAECARWK (SEQ ID NO: 285);  
EAKLLECARAR (SEQ ID NO: 286);  
ELSLCEARAGVCAGSVTK (SEQ ID NO: 287);  
ELSLCEAKAGVCAGSVTK (SEQ ID NO: 288);  
ALWQCVARLEECARSR (SEQ ID NO: 289);  
20 CLKSCSELKLDECARRM (SEQ ID NO: 290);  
ALQTCEWRLQECARSR (SEQ ID NO: 291);  
YISQCEAKLAECARLY (SEQ ID NO: 292);  
ELSSCEAKLSECARRW (SEQ ID NO: 293);  
ELSSCEARLSECARRW (SEQ ID NO: 294);  
25 QLLQCELKLLECARQG (SEQ ID NO: 295);  
ELLRCEARLAECARGC (SEQ ID NO: 296);  
QLRQCELRLQECGRHGN (SEQ ID NO: 297);  
PLTSCEQKLAECARRF (SEQ ID NO: 298);  
LLGMCELRLQECARAK (SEQ ID NO: 299);  
30 ELSRCELKLEECARGM (SEQ ID NO: 300);  
DCRPCESRLEECARRL (SEQ ID NO: 301);  
RLSVCEARLEECARQL (SEQ ID NO: 302);

PLKMCEATVQECARLI (SEQ ID NO: 303);  
LLLFCEARLSECARHV (SEQ ID NO: 304);  
SLSMCEARLAECARLL (SEQ ID NO: 305);  
PLFSCSELKLQECARRCN (SEQ ID NO: 306);  
5 SLERCYSRLEECGRRI (SEQ ID NO: 307);  
PLTSCSELRLRECALRSN (SEQ ID NO: 308);  
KLAACELKLAECARRW (SEQ ID NO: 309);  
KLAACELRLAECARRW (SEQ ID NO: 310);  
ALTRCELRLAECARKI (SEQ ID NO: 311);  
10 LLQQCELKLAECARSI (SEQ ID NO: 312);  
QLWQCEARLLECARRS (SEQ ID NO: 313);  
RLRLCESRLLECARS (SEQ ID NO: 314);  
QLETCVLKLEECARRCN (SEQ ID NO: 315);  
ALSQCELRLAECARSVTK (SEQ ID NO: 316);  
15 ELKLAECARRS (SEQ ID NO: 317);  
ALSRCESKLAECARRQ (SEQ ID NO: 318);  
LMSTCESKLRECARSL (SEQ ID NO: 319);  
SLQRCEYKLGECARS (SEQ ID NO: 320);  
RLELLESRLQECARQLN (SEQ ID NO: 321);  
20 QMEWCQARLAECARCCN (SEQ ID NO: 322);  
PLFSCSELKKQECARRCN (SEQ ID NO: 323);  
LLDKCESRLSECARRL (SEQ ID NO: 324);  
LLARCEARLEECGRQC (SEQ ID NO: 325);  
DLLYCESRLAECGRM (SEQ ID NO: 326);  
25 ALQMCEWRLEECARRL (SEQ ID NO: 327);  
LLTMCEARLSECGRRL (SEQ ID NO: 328);  
ALWRCESRLAECARRS (SEQ ID NO: 329);  
LLATCAARLAECARQL (SEQ ID NO: 330);  
LQTCEWKLAECARSN (SEQ ID NO: 331);  
30 PLRSCESKLEECARQL (SEQ ID NO: 332);  
CLRALDVKLAECARHL (SEQ ID NO: 333);  
RLKTLELQLEECARRS (SEQ ID NO: 334);

KLRDVELKLAECARRS (SEQ ID NO: 335);  
 SLQRCYKLAECARRS (SEQ ID NO: 336);  
 RLARCELRLAECARRS (SEQ ID NO: 337);  
 DLWYLESKLEECARRCN (SEQ ID NO: 338);  
 5 DLWYLESKLEECARRANG (SEQ ID NO: 339);  
 DLWYLESKLEECARRCNG (SEQ ID NO: 340);  
 KQRELELKLAECARRS (SEQ ID NO: 341);  
 QMQEWCARLAECARCCN (SEQ ID NO: 342); and  
 LLDICELKLQECARRAN (SEQ ID NO: 343).

10

A particularly preferred sequence of formula (VI) is:

LLDICELKLQECARRAN (SEQ ID NO: 343).

#### G. COMPOUNDS OF FORMULA (VII):

15

In another aspect, the invention provides compounds comprising a peptide chain approximately 10 to 40 amino acids in length that binds to G-CSFR and contains a sequence of amino acids of formula (VII):

(VII)  $X^{VI}_1X^{VI}_2X^{VI}_3X^{VI}_4X^{VI}_5EX^{VI}_6X^{VI}_7X^{VI}_8X^{VI}_9$  (SEQ ID NO: 7)

20 wherein each amino acid is indicated by standard one-letter abbreviation, and wherein  $X^{VI}_1$  is A, E or G;  $X^{VI}_2$  is E, H or D;  $X^{VI}_3$  is R or G;  $X^{VI}_4$  is K, Y, M, N, Q, R, D, I, S or E;  $X^{VI}_5$  is A, S or P;  $X^{VI}_6$  is E, D, T, Q, K or A;  $X^{VI}_7$  is R, W, K, L, S, A or Q;  $X^{VI}_8$  is R or E; and  $X^{VI}_9$  is W, G, or R.

Preferably  $X^{VI}_1$  is A;  $X^{VI}_2$  is E;  $X^{VI}_3$  is R;  $X^{VI}_4$  is A;  $X^{VI}_5$  is E;  $X^{VI}_6$  is R;  $X^{VI}_7$  is R;  $X^{VI}_8$  is R; and  $X^{VI}_9$  is W.

25

Examples of particularly preferred sequences satisfying formula (VII) include, but are not limited to, the following:

AERKAEERRW (SEQ ID NO: 344);

AERYAEEREG (SEQ ID NO: 345);

AERMAEERRW (SEQ ID NO: 346);

30

AERKAEERRR (SEQ ID NO: 347);

AHRNAEERRW (SEQ ID NO: 348);

AERKSEDWRW (SEQ ID NO: 349);

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5 AERKAEKRR (SEQ ID NO: 350);  
AERQAETRRW (SEQ ID NO: 351);  
AERNAEERRW (SEQ ID NO: 352);  
AERQAEERRW (SEQ ID NO: 353);  
AERRAEERRW (SEQ ID NO: 354);  
AERDAEQRRW (SEQ ID NO: 355);  
AERIAEERRW (SEQ ID NO: 356);  
AERSAEERRW (SEQ ID NO: 357);  
AERKAEELRW (SEQ ID NO: 358);  
10 AERKAEESRW (SEQ ID NO: 359);  
EERKAEERRW (SEQ ID NO: 360);  
ADGKAEERRW (SEQ ID NO: 361);  
ADGKAEELRW (SEQ ID NO: 362);  
ADGMPEERRW (SEQ ID NO: 363);  
15 ADGEAEKRRW (SEQ ID NO: 364);  
ADGNAEERRW (SEQ ID NO: 365);  
ADGEAEKARW (SEQ ID NO: 366);  
AEGEAEKARW (SEQ ID NO: 367);  
GERKAEERRW (SEQ ID NO: 368);  
20 AEREAERRW (SEQ ID NO: 369);  
ADGEAEARRW (SEQ ID NO: 370);  
ADGRAEEARW (SEQ ID NO: 371);  
AEGRAEEARW (SEQ ID NO: 372);  
AEREA EKARW (SEQ ID NO: 373);  
25 AERKAEQRRW (SEQ ID NO: 374);  
AERDAEKRRW (SEQ ID NO: 375); and  
AEREA EKLRW (SEQ ID NO: 376).

Other preferred formula (VI) sequences include but are not limited to, the following:

30 MLAERKAEERRWFNTHGRE (SEQ ID NO: 377);  
MLAERKAEERRWFNTHGREK (SEQ ID NO: 378);  
GGGMLAERKAEERRWFNTHGRE (SEQ ID NO: 379);

CMLAERKAEERRWFNTHGRE (SEQ ID NO: 380);  
CMLAERKAEERRWFNTHGREK (SEQ ID NO: 381);  
MLAERYAEEREGFNMQWRE (SEQ ID NO: 382);  
MLAERMAEERRWFRRMG (SEQ ID NO: 383);  
5 IVAERKAEERRRLNTEGHE (SEQ ID NO: 384);  
ILAHRNAEERRWFQKHGR (SEQ ID NO: 385);  
MLAERKSEDWRWLKTHGRD (SEQ ID NO: 386);  
MLAERKAEERRLKTQGRE (SEQ ID NO: 387);  
ILAERQAETRRWMRNAGSVTK (SEQ ID NO: 388);  
10 MLAERNAEERRWLKRQCG (SEQ ID NO: 389);  
MLAERQAEERRWLKMHGGE (SEQ ID NO: 390);  
MLAERRAEERRWLKTQGGD (SEQ ID NO: 391);  
MLAERQAEERRWLKTQGRD (SEQ ID NO: 392);  
MLAERKAEERRWFKTHGRE (SEQ ID NO: 393);  
15 MLAERKAEERRWFNNQGRE (SEQ ID NO: 394);  
MPAERDAEQRRWLKTHGRE (SEQ ID NO: 395);  
ILAERIAEERRWLKTQGR (SEQ ID NO: 396);  
MLAERKAEERRWLQTHGRE (SEQ ID NO: 397);  
ILAERSAEERRWLKTQGRE (SEQ ID NO: 398);  
20 LLAERKAEELRWLKTHGRE (SEQ ID NO: 399);  
MLAERKAEERRWLQTHGRE (SEQ ID NO: 400);  
MLAERNAEERRW (SEQ ID NO: 401);  
MFAERKAEESRWLQSQGRE (SEQ ID NO: 402);  
MLEERKAEERRWLKTHGR (SEQ ID NO: 403);  
25 MLAERKAEERRWLKMQGRE (SEQ ID NO: 404);  
MLAERNAEERRWFYTHGRE (SEQ ID NO: 405);  
MLADGKAEERRWLKTHGLD (SEQ ID NO: 406);  
MLADGKAEERRWLKTHGRD (SEQ ID NO: 407);  
MLADGKAEELRWLKTQGS (SEQ ID NO: 408);  
30 MLAERNAEERRWLKTHGRD (SEQ ID NO: 409);  
MLADGKAEELRWLKTQGRE (SEQ ID NO: 410);  
ILADGKAEERRWLKTHGRD (SEQ ID NO: 411);



MLADGMPEERRWLQTHGRD (SEQ ID NO: 412);  
MLADGEAEKRRWLNTHGRD (SEQ ID NO: 413);  
MLADGNAEERRWLMTHGRD (SEQ ID NO: 414);  
MLADGEAEKARWLKTQGRE (SEQ ID NO: 415);  
5 MLAEGEAEKARWLKTQGRE (SEQ ID NO: 416);  
MLADGKAEERRWLKTQGRE (SEQ ID NO: 417);  
MLAERKAEERRWLSAHVRE (SEQ ID NO: 418);  
LLGERKAEERRWYKTHARE (SEQ ID NO: 419);  
MLAERKAEERRWLMTHGHD (SEQ ID NO: 420);  
10 MLAERKAEERRWLKSQCLE (SEQ ID NO: 421);  
LLAEREAEEERRWFKTHGRE (SEQ ID NO: 422);  
MLADGEAEARRWFNMHGRE (SEQ ID NO: 423);  
MLADGRAEEARWLKTQGSE (SEQ ID NO: 424);  
MLAEGRAEEARWLKTQGSE (SEQ ID NO: 425);  
15 MLAEREA EKARWLKTQGRE (SEQ ID NO: 426);  
MMAERKAEEQRWFDIHGRD (SEQ ID NO: 427);  
LTAERDAEKRRWLLTHGGE (SEQ ID NO: 428);  
MLAERQAEERRWLKSQRGE (SEQ ID NO: 429);  
LLAERKAEERRWFATHGRD (SEQ ID NO: 430);  
20 MLAEREA EKLRWLKSQERA (SEQ ID NO: 431);  
MLAERKAEERRWLKTHGGE (SEQ ID NO: 432);  
KGGGMLAERKAEERRWFNTHGRE (SEQ ID NO: 490); and  
KSTGGLTAERDAEKRRWLLTHGGE (SEQ ID NO: 491).

## 25 H. OTHER ACTIVE COMPOUNDS

In another aspect of the invention, there are provided additional compounds comprising a peptide chain approximately 5 to 40 amino acids in length that binds to G-CSFR and contains a sequence of amino acids selected from the following compounds:

CTWTDLESVY (SEQ ID NO: 433);  
30 HTTNEQFFMC (SEQ ID NO: 434);  
DTWLELESRY (SEQ ID NO: 435);  
HNSSPMVGVT (SEQ ID NO: 436);

DWQKTPAYW (SEQ ID NO: 437);  
RWGREGLVAALL (SEQ ID NO: 438);  
WSGTRVWRCVVT (SEQ ID NO: 439);  
MSLLSYLRS (SEQ ID NO: 440);  
5 LDLLAI (SEQ ID NO: 441);  
RIYGVK (SEQ ID NO: 442);  
MIWHMFMSLLF (SEQ ID NO: 443);  
FFWASWMHLLW (SEQ ID NO: 444);  
FDDCWREREQFLFQAL (SEQ ID NO: 445);  
10 CGRASECFRLLEM (SEQ ID NO: 446);  
RECFQMLER (SEQ ID NO: 447);  
CSIRWDFVPGYGLC (SEQ ID NO: 448);  
WMQCWDSLRLCYDM (SEQ ID NO: 449);  
ALLMCESKLAECARAR (SEQ ID NO: 450);  
15 LAHCKKRKEECAAG (SEQ ID NO: 451);  
SIDGVYLRTSRT (SEQ ID NO: 452);  
SIDGVYLRTRSRTY (SEQ ID NO: 453);  
VRWLRGSTLRGLRDR (SEQ ID NO: 454);  
DRGGGTGVGYWWESY (SEQ ID NO: 455);  
20 VWGTVGTWLEY (SEQ ID NO: 456);  
LMWVSAY (SEQ ID NO: 457);  
RASDEYGALVRFCTNL (SEQ ID NO: 458);  
NYWCDSNWVCEIA (SEQ ID NO: 459);  
LAHCLLRLEECAAG (SEQ ID NO: 460);  
25 LALCLARLRECAGG (SEQ ID NO: 461);  
CESRLVECSRM (SEQ ID NO: 462);  
LLDIAELKLQECARRCN (SEQ ID NO: 463);  
KLLDIAELKLQECARRCN (SEQ ID NO: 464);  
CSTGGGLTAERDAEKRRWLLTHGGE (SEQ ID NO: 465);  
30 LTAERDAEKRRWLLTHGGEGG (SEQ ID NO: 466);  
LTAERDAEKRRWLLTHGGEGGK (SEQ ID NO: 467);  
LTAERDAEKRRWLLTHGGEGGGG (SEQ ID NO: 468);

LTAERDAEKRRWLLTHGGEGGGGGK (SEQ ID NO: 469);  
ESGWVW (SEQ ID NO: 470);  
NSGWVW (SEQ ID NO: 471);  
SGWVW (SEQ ID NO: 472);  
5 PLGKCEATCREMARYFN (SEQ ID NO: 473);  
SLQRCEYKLASVRGLCN (SEQ ID NO: 474)  
DLWYLESKLEEAARRCNG (SEQ ID NO: 475);  
PYMGTRSRAKLLRQQ (SEQ ID NO: 476);  
RNAGERRWFKTQGWY (SEQ ID NO: 477);  
10 MLAERNADDRRWFNTHGRD (SEQ ID NO: 478);  
MMADGRLRNSVGLILWCD (SEQ ID NO: 479);  
MLADGRLRNVVG (SEQ ID NO: 480);  
LLADVRRRNGVGLLRMGRD (SEQ ID NO: 481);  
MLADGRLRNFGG (SEQ ID NO: 482);  
15 TYMTYVYWLC (SEQ ID NO: 483);  
RFGERWGL (SEQ ID NO: 484);  
HWLWWGWNF (SEQ ID NO: 485);  
RECFQMLERC (SEQ ID NO: 486);  
ILAHRNAKERRWFQKHGR (SEQ ID NO: 487); and  
20 CSTGGGLTAERDAEKRRWLLTHGGEK (SEQ ID NO: 489).

Particularly preferred sequences are selected from the group consisting of:

LLDIAELKLQECARRCN (SEQ ID NO: 463); and  
KLLDIAELKLQECCARRCN (SEQ ID NO: 464).

25

#### **I. SYNTHESIS OF THE PEPTIDES:**

Standard solid phase peptide synthesis techniques are preferred for synthesis of the peptides of the present invention. Such techniques are described, for example, by Merrifield (1963) *J. Am. Chem. Soc.* 85:2149. As is well known in the art, solid phase  
30 synthesis using the Merrifield method involves successive coupling of  $\alpha$ -amino protected amino acids to a growing support-bound peptide chain. After the initial coupling of a protected amino acid to a resin support (e.g., a polystyrene resin, a chloromethylated resin,

a hydroxymethyl resin, a benzhydrylamine resin, or the like, depending on the chemistry used), the  $\alpha$ -amino protecting group is removed by a choice of reagents, depending on the specific protecting group. Suitable  $\alpha$ -amino protecting groups are those known to be useful in the art of stepwise synthesis of peptides. Included are acyl type protecting groups (e.g., formyl, trifluoroacetyl, acetyl), aromatic urethane type protecting groups (e.g., benzyloxycarbonyl (Cbz) and substituted Cbz), aliphatic urethane protecting groups (e.g., t-butyloxycarbonyl (Boc), isopropylloxycarbonyl, cyclohexyloxycarbonyl), alkyl type protecting groups (e.g., benzyl, triphenylmethyl), fluorenylmethyl oxycarbonyl (Fmoc), alloxycarbonyl (Alloc) and Dde. The side chain protecting groups (typically ethers, esters, trityl, and the like) remain intact during coupling; however, the side chain protecting group must be removable upon completion of the synthesis of the final peptide. Preferred side chain protecting groups, as will be appreciated by those skilled in the art, will depend on the particular amino acid that is being protected as well as the overall chemistry used. After removal of the  $\alpha$ -amino protecting group, the remaining protected amino acids are coupled stepwise in the desired order. Each protected amino acid is generally reacted in about a 3-fold excess using an appropriate carboxyl group activator such as 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) or dicyclohexylcarbodiimide (DCC) in solution, for example, in methylene chloride ( $\text{CH}_2\text{Cl}_2$ ), N-methyl pyrrolidone, dimethyl formamide (DMF), or mixtures thereof.

Once the synthesis is complete, the compound is cleaved from the solid support by treatment with a reagent such as trifluoroacetic acid, preferably in combination with a scavenger such as ethanedithiol,  $\beta$ -mercaptoethanol or thioanisole. The cleavage reagent not only cleaves the peptide from the resin, but also cleaves all remaining side chain protecting groups.

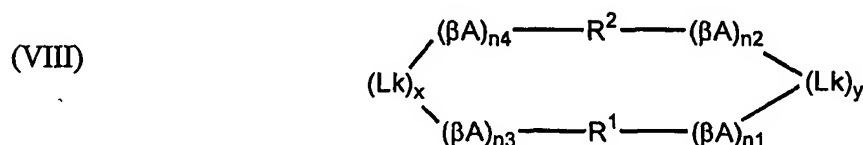
These procedures can also be used to synthesize peptides containing amino acids other than the 20 naturally occurring, genetically encoded amino acids. For instance, naphthylalanine can be substituted for tryptophan, with 1-Nal or 2-Nal. Other synthetic amino acids that can be substituted into the peptides of the present invention include, but are not limited to, nor-leucine and 3-pyridylalanine.

### III. VARIATIONS AND MODIFICATIONS

#### A. DIMER FORMS (WITH A TERMINAL LINKING MOIETY):

The compounds of the present invention may be in the form of a dimer, i.e., a compound comprised of two similar (but not necessarily identical) peptide sequences.

5 Preferably, the dimer compounds of the invention have the structure of formula (VIII)



10

wherein  $R^1$ ,  $R^2$ ,  $n1$ ,  $n2$ ,  $n3$ ,  $n4$ ,  $x$ ,  $y$  and  $Lk$  are defined as follows.

$R^1$  is a peptide chain that binds to G-CSFR and contains a sequence of amino acids of the present invention.  $R^2$  is also a peptide chain that binds to G-CSFR and contains a sequence of amino acids of the present invention. As previously indicated,  $R^1$  and  $R^2$  can be the same or different. It is preferred, however, that  $R^1$  and  $R^2$  are the same.

15

$\beta A$  is a  $\beta$ -alanine residue and may or may not be present, meaning that  $n1$ ,  $n2$ ,  $n3$  and  $n4$  are independently zero or 1.

$Lk$  is a terminal linking moiety. If the dimer contains only one linking moiety, one of  $x$  and  $y$  is zero and the other is one. Alternatively, if the dimer contains two linking moieties, both  $x$  and  $y$  are one. Thus,  $x$  and  $y$  are independently zero or one with the proviso that the sum of  $x$  and  $y$  is either one or two.

20

The terminal linking moiety  $Lk$  can be any moiety recognized by those skilled in the art as suitable for joining the peptides of  $R^1$  and  $R^2$ .  $Lk$  is preferably although not necessarily selected from the group consisting of a disulfide bond, a carbonyl moiety and a  $C_{1-12}$  linking moiety optionally terminated with one or two -NH- linkages and optionally substituted at one or more available carbon atoms with a lower alkyl substituent.

25

Preferably, the terminal linking moiety comprises -NH- $R^3$ -NH- wherein  $R^3$  is lower ( $C_{1-6}$ ) alkylene substituted with a functional group such as a carboxyl group or an amino group that enables coupling to another molecular moiety (e.g., as may be present on the surface of a solid support), and is optionally substituted with a lower alkyl group. Optimally, the

30

linking moiety is a lysine residue or lysine amide, i.e., a lysine residue wherein the carboxyl group has been converted to an amide moiety  $-\text{CONH}_2$ .

5  $\text{NH}_2\text{-EQSNSGWVWVG GGC-CONH}_2$  (SEQ ID NO: 101)  
 $\text{NH}_2\text{-EQSNSGWVWVG GGC-CONH}_2$  (SEQ ID NO: 101);

10  $\text{CSTGGGLTAERDAEKRRWLLTHGGE}$  (SEQ ID NO: 465)  
 $\text{CSTGGGLTAERDAEKRRWLLTHGGE}$  (SEQ ID NO: 489);

15  $\text{MLAERKAEERRWFNTHGRE}$  (SEQ ID NO: 377)  
 $\text{MLAERKAEERRWFNTHGRE-K(NH}_2\text{)}$  (SEQ ID NO: 378);

$\text{CMLAERKAEERRWFNTHGRE}$  (SEQ ID NO: 380)  
 $\text{CMLAERKAEERRWFNTHGRE-K}$  (SEQ ID NO: 381);

20  $\text{LTAERDAEKRRWLLTHGGEGG}$  (SEQ ID NO: 466)  
 $\text{LTAERDAEKRRWLLTHGGEGG-K}$  (SEQ ID NO: 467); and

25  $\text{LTAERDAEKRRWLLTHGGEGGGGG}$  (SEQ ID NO: 468)  
 $\text{LTAERDAEKRRWLLTHGGEGGGGG-K}$  (SEQ ID NO: 469).

#### B. DISULFIDE BONDS:

30 When a pair of cysteine residues is present in a peptide of the invention, it is preferred that the pair form a disulfide bond linking these residues. The disulfide bond may be present within a single peptide chain forming an intramolecular disulfide bond. Alternatively, if the compound includes an additional cysteine-containing peptide chain, the disulfide bond may connect the two chains. In addition, where an additional pair of  
 35 cysteine residues exists in the compound, more than one disulfide bond may be present.

Disulfide bond formation may be effected by techniques well known to those skilled in the art. One such technique involves employing a suitable oxidizing reagent

such that a disulfide bond forms from the free thiols from a pair of cysteine residues. Undesired disulfide bond formation can be minimized, for example, by protecting the thiol groups of those cysteine residues not intended to form disulfide bonds and oxidizing the peptide before removal of any protecting groups. Preferred compounds having disulfide bonds include, by way of example, the following:

NH<sub>2</sub>-STAERLWFCG-CONH<sub>2</sub> (SEQ ID NO: 135)

NH<sub>2</sub>-STAERLWFCG-CONH<sub>2</sub> (SEQ ID NO: 135);

NH<sub>2</sub>-QNSEREWFC-CONH<sub>2</sub> (SEQ ID NO: 138)

NH<sub>2</sub>-QNSEREWFC-CONH<sub>2</sub> (SEQ ID NO: 138);

NH<sub>2</sub>-QNSEREWFCG-CONH<sub>2</sub> (SEQ ID NO: 149)

NH<sub>2</sub>-QNSEREWFCG-CONH<sub>2</sub> (SEQ ID NO: 149);

[H]-DLWYLESKLEECARRANG-[NH<sub>2</sub>] (SEQ ID NO: 339)

[H]-DLWYLESKLEECARRANG-[NH<sub>2</sub>] (SEQ ID NO: 339);

[H]-DLWYLESKLEEAARRCNG-[NH<sub>2</sub>] (SEQ ID NO: 475)

[H]-DLWYLESKLEEAARRCNG-[NH<sub>2</sub>] (SEQ ID NO: 475);

[H]-DLWYLESKLEECARRCNG-[NH<sub>2</sub>] (SEQ ID NO: 340);

[H]-LLDICEKLQECARRAN-[OH] (SEQ ID NO: 343);

[H]-LLDICEKLQEAARRCN-[OH] (SEQ ID NO: 212);

[H]-K-LLDICEKLQEAARRCN-[OH] (SEQ ID NO: 231);

[Biotin]

[H]-LLDIAELKLQECARRCN-[OH] (SEQ ID NO: 463);

[H]-KLLDIAELKLQECARRCN-[OH] (SEQ ID NO: 464); and

NH<sub>3</sub><sup>+</sup>-LLDICE LKLQECARRCN-COO<sup>-</sup> (SEQ ID NO: 208)

                  |                  |                  |  
NH<sub>3</sub><sup>+</sup>-LLDICE LKLQECARRCN-COO<sup>-</sup> (SEQ ID NO: 208).

5 A particularly preferred compound having disulfide bonds includes

NH<sub>3</sub><sup>+</sup>-LLDICE LKLQECARRCN-COO<sup>-</sup> (SEQ ID NO: 208)

                  |                  |                  |  
NH<sub>3</sub><sup>+</sup>-LLDICE LKLQECARRCN-COO<sup>-</sup> (SEQ ID NO: 208).

10

### C. N-TERMINAL MODIFICATIONS:

#### (i) PEGYLATED COMPOUNDS

The peptides and compounds of the invention can advantageously be modified  
15 with or covalently coupled to one or more of a variety of hydrophilic polymers. It has been  
found that when the peptide compounds are derivatized with a hydrophilic polymer, their  
solubility and circulation half-lives are increased and their immunogenicity is masked.  
Quite surprisingly, the foregoing can be accomplished with little, if any, diminishment in  
binding activity. Nonproteinaceous polymers suitable for use in accordance with the  
20 present invention include, but are not limited to, polyalkylethers as exemplified by  
polyethylene glycol and polypropylene glycol, polylactic acid, polyglycolic acid,  
polyoxyalkenes, polyvinylalcohol, polyvinylpyrrolidone, cellulose and cellulose  
derivatives, dextran and dextran derivatives, etc. Generally, such hydrophilic polymers  
have an average molecular weight ranging from about 500 to about 100,000 daltons, more  
25 preferably from about 2,000 to about 60,000 daltons and, even more preferably, from  
about 5,000 to about 50,000 daltons. In preferred embodiments, such hydrophilic  
polymers have average molecular weights of about 5,000 daltons, 10,000 daltons 20,000  
daltons and 40,000 daltons.

The peptide compounds of the invention can be derivatized with or coupled to  
30 such polymers using any of the methods set forth in Zallipsky (1995) *Bioconjugate Chem.*  
6:150-165; Monfardini et al. (1995) *Bioconjugate Chem.* 6:62-69; U.S. Patent No.  
4,640,835; U.S. Patent No. 4,496,689; U.S. Patent No. 4,301,144; U.S. Patent No.  
4,670,417; U.S. Patent No. 4,791,192; U.S. Patent No. 4,179,337 or WO 95/34326.

In a preferred embodiment, the N-terminus of a peptide of the invention is  
35 coupled to a polyethylene glycol molecule. It is particularly preferred that the polymer is



selected from the group consisting of polyethylene glycol, polypropylene glycol, polylactic acid, polyglycolic acid and derivatives and combinations thereof. Most preferably the polymer is polyethylene glycol (PEG), in which case the peptide is referred to as "PEGylated." PEG is a linear, water-soluble polymer of ethylene oxide repeating units with two terminal hydroxyl groups. PEGs are classified by their molecular weights which typically range from about 500 daltons to about 40,000 daltons. In a presently preferred embodiment, the PEGs employed have an average molecular weight of from about 500 to about 80,000 daltons. It is particularly preferred that the polymer has an average molecular weight of between about 5,000 to 40,000 daltons.

The PEG coupled to the peptide compounds of the invention can be either branched or unbranched. (See, e.g. Monfardini et al. (1995) *Bioconjugate Chem.* 6:62-69.) PEG is commercially available from Shearwater Polymers, Inc. (Huntsville, Alabama), Sigma Chemical Co. and other companies. Suitable PEGs include, but are not limited to, monomethoxypolyethylene glycol (MePEG-OH), monomethoxypolyethylene glycol-succinate (MePEG-S), monomethoxypolyethylene glycol-succinimidyl succinate (MePEG-S-NHS), monomethoxypolyethylene glycol-amine (MePEG-NH<sub>2</sub>), monomethoxypolyethylene glycol-tresylate (MePEG-TRES) and monomethoxypolyethylene glycol-imidazolyl-carbonyl (MePEG-IM).

Briefly, in one exemplary embodiment, the hydrophilic polymer which is employed, e.g., PEG, is capped at one terminus by an unreactive group such as a methoxy or ethoxy group. Thereafter, the polymer is activated at the other terminus by reaction with a suitable activating agent, such as a cyanuric halide (e.g., cyanuric chloride, bromide or fluoride), diimidazole, an anhydride reagent (e.g., a dihalosuccinic anhydride, such as dibromosuccinic anhydride), acyl azide, *p*-diazoniumbenzyl ether, 3-(*p*-diazoniumphenoxy)-2-hydroxypropylether, or the like. The activated polymer is then reacted with a peptide compound of the invention to produce a polymer-derivatized peptide compound. Alternatively, a functional group in the peptide compounds of the invention can be activated for reaction with the polymer, or two groups can be joined in a concerted coupling reaction using known coupling methods. It will be readily appreciated that the peptide compounds of the invention can be derivatized with PEG using a myriad of other reaction schemes known to those of skill in the art.

**(ii) ACETYLATED COMPOUNDS**

In some instances, the N-terminus of the peptide is acetylated. Preferred acetylated compounds include, by way of example, the following:

Ac-ESGWVW-CONH<sub>2</sub> (SEQ ID NO: 470);

5 Ac-NSGWVW-CONH<sub>2</sub> (SEQ ID NO: 471); and

Ac-SGWVW-CONH<sub>2</sub> (SEQ ID NO: 472).

The peptides and compounds of the invention can be modified with an acetyl moiety (Ac) using standard techniques known to those skilled in the art. One such technique includes combining the peptide with an acetylating reagent (e.g., acetyl chloride, 10 acetic anhydride) in a suitable solvent to form the acetylated product. To the extent that other acetylated products are formed during the reaction, the N-terminus derivative can be isolated using conventional separation techniques.

**D. C-TERMINAL MODIFICATIONS:**

15 The peptides and compounds of the invention can advantageously be modified to include an amide functionality at the carboxyl terminus of the peptide. Thus, it is preferred that the C-terminus of the peptide is amidated.

In preparing peptides wherein the C-terminus carboxyl group is replaced by the amide -C(O)NR<sup>3</sup>R<sup>4</sup> where R<sup>3</sup> and R<sup>4</sup> are independently H or lower (C<sub>1-6</sub>) alkyl, a 20 benzhydrylamine resin is preferably used as the solid support for peptide synthesis. Upon completion of the synthesis, a hydrogen fluoride treatment is employed to release the peptide from the support, directly resulting in the free peptide amide (i.e., the C-terminus is -C(O)NH<sub>2</sub>). Alternatively, use of a chloromethylated resin during peptide synthesis coupled with reaction with ammonia (to cleave the side chain protected peptide from the 25 support) yields the free peptide amide and reaction with an alkylamine or a dialkylamine yields a side chain protected alkylamide or dialkylamide (i.e., the C-terminus is -C(O)NR<sup>3</sup>R<sup>4</sup> where R<sup>3</sup> and R<sup>4</sup> are as defined above). Side chain protecting groups are then removed in the usual fashion by treatment with hydrogen fluoride to give the free amides, alkylamides, or dialkylamides.

#### E. OTHER MODIFICATIONS:

One can also replace the naturally occurring side chains of the 20 genetically encoded amino acids (or the stereoisomeric D amino acids) with other side chains, for instance with groups such as alkyl, lower alkyl, cyclic 4-, 5-, 6- or 7-membered alkyl, amide, amide lower alkyl, amide di(lower alkyl), lower alkoxy, hydroxy, carboxy and the lower ester derivatives thereof, and 4-, 5-, 6- or 7-membered heterocyclic. In particular, proline analogues in which the ring size of the proline residue is changed from 5 members to 4, 6, or 7 members can be employed.

One can also readily modify the peptides herein by phosphorylation or other methods as described in Hruby et al. (1990) *Biochem J.* 268:249-262. Thus, the peptides of the invention also serve as structural models for non-peptidic compounds with similar biological activity. For example, the peptide backbones may be replaced with a backbone composed of phosphonates, amidates, carbamates, sulfonamides, secondary amines, and N-methylamino acids.

#### IV. UTILITY

The compounds of the invention are useful *in vitro* as unique tools for understanding the biological role of G-CSF, including the evaluation of the many factors thought to influence, and be influenced by, the production of white blood cells. The present compounds are also useful in the development of other compounds that bind to G-CSFR, because the compounds provide important structure-activity relationship (SAR) information that facilitates that development.

Moreover, based on the ability to bind to G-CSFR and related receptors, a compound of the invention can be used as a reagent for detecting a G-CSF receptor or related receptor on living cells, fixed cells, in biological fluids, in tissue homogenates, in purified, natural biological materials, etc. For example, by labeling a compound of the invention, one can identify a cell expressing G-CSFR on its surface. In addition, based on its ability to bind a G-CSFR, a compound of the invention can be used in *in situ* staining, FACS (fluorescence-activated cell sorting), Western blotting, ELISA (enzyme-linked immunoadsorptive assay), etc. In addition, because of its ability to bind to a G-CSFR, a compound of the invention can be used in receptor purification or in purifying cells expressing G-CSFR on the cell surface (or inside permeabilized cells).

A compound of the invention can also be utilized as a commercial research reagent for various medical research and diagnostic uses. Such uses include but are not limited to: (1) use as a calibration standard for quantitating the activities of candidate G-CSFR antagonists or agonists in a variety of functional assays; (2) use as a blocking  
5 reagent in random peptide screening, i.e., in searching for new families of G-CSFR peptide ligands; (3) use in the co-crystallization with G-CSFR, i.e., a compound of the invention will allow formation of crystals bound to G-CSFR, enabling the determination of receptor/peptide structure x-ray crystallography; (4) use in inhibiting or decreasing the proliferation and growth of G-CSF-dependent cell lines; and (5) other research and  
10 diagnostic applications wherein the action of G-CSFR is to be mimicked, and the like.

A compound of the invention can also be administered to a warm blooded animal, including a human, to treat a disease that would benefit from the ability of a compound to mimic the effects of G-CSF *in vivo*. Thus, the present invention encompasses methods for treating a patient who would benefit from a G-CSFR modulator, comprising  
15 administering to the patient a therapeutically effective amount of a compound of the invention to activate G-CSFR. For example, a compound of this invention will find use in the treatment of diseases such as a depressed neutrophil count. Although attributable to a myriad of causes, a depressed neutrophil count is commonly associated with chemotherapy, AIDS and pneumonia (particularly community-acquired pneumonia).  
20 Thus, it is preferred that a compound of the present invention be used to treat a depressed neutrophil count selected from the group consisting of chemotherapy-induced neutropenia, AIDS-induced neutropenia and community-acquired pneumonia-induced neutropenia.

In addition, the invention encompasses methods for treating a patient who would benefit from a G-CSFR modulator, comprising administering to the patient a  
25 therapeutically effective amount of a compound of the invention that antagonizes the action of G-CSF to the G-CSFR *in vivo*. For example, these receptor antagonists are administered prior to and during chemotherapy to confer chemoprotection to the neutrophil progenitor cells by preventing their proliferation in the presence of cytotoxic drugs. Once chemotherapy administration is suspended, the administration of the chemoprotective G-  
30 CSFR antagonists is also suspended thereby allowing the patient's endogenous G-CSF to stimulate proliferation. Alternatively, the neutrophil progenitor cells may be "rescued" by

administration of G-CSF or by a G-CSF agonist, e.g., a compound of the present invention having G-CSF agonist activity.

Accordingly, the invention includes pharmaceutical compositions comprising, as an active ingredient, at least one of the compounds of the invention in association with a pharmaceutical carrier or diluent. The composition can be administered by oral, parenteral (intramuscular, intraperitoneal, intravenous (IV) or subcutaneous) injection, transdermal (either passively or using iontophoresis or electroporation), or transmucosal (nasal, vaginal, rectal, or sublingual) routes of administration, or using bioerodible inserts, and can be formulated in dosage forms appropriate for each route of administration.

10 Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound is admixed with at least one inert pharmaceutically acceptable carrier such as sucrose, lactose, or starch. Such dosage forms can also comprise, as is normal practice, an additional substance other than an inert diluent, e.g., a lubricating agent such as magnesium stearate. In the case of capsules, tablets, and pills, the dosage forms may also comprise a buffering agent. Tablets and pills can additionally be prepared with enteric coatings.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions and syrups, with the elixirs containing an inert diluent commonly used in the art, such as water. These compositions can also include one or more adjuvants, such as a wetting agent, an emulsifying agent, a suspending agent, a sweetening agent, a flavoring agent or a perfuming agent.

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents or vehicles are propylene glycol, polyethylene glycol, vegetable oils, such as olive oil and corn oil, gelatin, and injectable organic esters such as ethyl oleate. Such dosage forms may also contain one or more adjuvants such as a preserving agent, a wetting agent, an emulsifying agent and a dispersing agent. The dosage forms may be sterilized by, for example, filtration through a bacteria-retaining filter, by incorporating sterilizing agents into the compositions, by irradiating the compositions, or by heating the compositions. They can also be manufactured using sterile water, or some other sterile injectable medium, prior to use.

Compositions for rectal or vaginal administration are preferably suppositories which may contain, in addition to the active substance, an excipient such as cocoa butter or a suppository wax. Compositions for nasal or sublingual administration are also prepared with one or more standard excipients well known in the art.

5           The dosage of active ingredient in the compositions of this invention may be varied; however, it is necessary that the amount of the active ingredient is such that a suitable dosage form is obtained. The selected dosage depends upon the desired therapeutic effect, the route of administration, the duration of the treatment desired, and other factors well known to those skilled in the art. Generally, dosage levels of between  
10   0.001 to 10 mg/kg of body weight daily are administered to mammals.

          It is to be understood that while the invention has been described in conjunction with the preferred specific embodiments thereof, that the foregoing description as well as the examples which follow are intended to illustrate and not limit the scope of the invention. Other aspects, advantages and modifications within the scope of the invention  
15   will be apparent to those skilled in the art to which the invention pertains.

## **EXPERIMENTAL**

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to prepare and use the compounds disclosed and claimed herein. Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.) but some errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in °C and pressure is at or near atmospheric.

Standard peptide synthetic methods were used, and solid phase reactions were carried out at room temperature. Unless otherwise indicated, all starting materials and reagents were obtained commercially, e.g., from Aldrich, Sigma and ICN, and used without further purification. Standard cell culture and cell harvesting procedures were used.

Also, in these examples and throughout this specification, the abbreviations employed have their generally accepted meanings, as follows:

Ac = acetyl

BSA = bovine serum albumin

DMSO = dimethyl sulfoxide

DTT = dithiothreitol

HPLC = high pressure liquid chromatography

MBP = maltose binding protein

PBS = phosphate-buffered saline

SDS PAGE = sodium dodecyl sulfate polyacrylamide gel electrophoresis

TCEP = tris(2-carboxyethyl) phosphine

TFA = trifluoroacetic acid

Tris = tris[hydroxymethyl]aminomethane

## **EXAMPLES 1-34**

### **G-CSF COMPETITION BINDING ASSAYS**

The peptides of Table 1 were synthesized using standard techniques and were subsequently evaluated to identify whether the peptides exhibited specific and/or competitive binding.

Specific binding is binding of a ligand to a specific receptor, as opposed to

non-specific binding that is mediated by non-specific interactions. Specific binding may be measured by subtraction of the non-specific binding (measured in the presence of saturating concentrations of unlabeled ligand) from the total binding (measured in the absence of saturating amounts of ligand). Typically, the unlabeled ligand used was a variant of G-CSF in which the cysteine normally found at position 17 was converted to serine (CS17).

Determination of competitive binding was also carried out for a number of peptides. Briefly stated, G-CSFR was purified using standard techniques. The receptor was then immobilized in microtiter plate wells that were coated with acid-treated antibody (Ab179) specific for a site on G-CSFR not involved with G-CSF binding. Separately,  $^{125}\text{I}$  was coupled to the natural ligand G-CSF using techniques well known in the art. Test peptides were added to receptor-coated wells and allowed to bind to immobilized receptor for approximately 30 minutes.  $^{125}\text{I}$  labeled G-CSF was then introduced to the wells and incubated overnight at 4 °C. Unbound  $^{125}\text{I}$  labeled G-CSF was removed by washing the plate several times followed by measuring the amount of radioactivity that remained in each well using conventional techniques. If no reduction in the amount of bound  $^{125}\text{I}$  labeled G-CSF was detected, the peptide did not compete for binding to the receptor. Alternatively, if reduced amounts or no  $^{125}\text{I}$  labeled G-CSF was detected, the peptide did compete. Non-positive results for a particular peptide are not dispositive of that peptide's activity: the peptide may exhibit binding under conditions different from those tested.

The results of these assays reveal important information about the structure activity relationship for peptide and peptide mimetics of the invention to the G-CSF receptor.

Table 1

Ex. No.	Sequence	Specific Binding ?	Competitive Binding ?
1	CAGEVMHMCC (SEQ ID NO: 8)	Yes	Yes
2	CNREIEAMCC (SEQ ID NO: 9)	Yes	Yes
3	CADEVMHFCC (SEQ ID NO: 10)	Yes	Yes
4	CDVWQLFDRC (SEQ ID NO: 25)	Yes	Yes
5	CSFVQLNSIC (SEQ ID NO: 26)	Yes	Yes
6	CVPWMFYDLC (SEQ ID NO: 29)	Yes	No



Ex. No.	Sequence	Specific Binding ?	Competitive Binding ?	
7	CDPWMFYDLC (SEQ ID NO: 30)	Yes	No	
8	CQRAGYMLAC (SEQ ID NO: 44)	No	No	
9	CHANPVWGEC (SEQ ID NO: 45)	No	No	
10	CTWTDLESVY (SEQ ID NO: 433)	No	No	
5	11	CFWSDWGQTC (SEQ ID NO: 46)	No	No
	12	CPDWYQSYMC (SEQ ID NO: 34)	Yes	Yes
	13	CPHWTSYYMC (SEQ ID NO: 47)	Yes	Yes
	14	CACMLRVVHC (SEQ ID NO: 43)	Yes	Yes
	15	CETLCGACFC (SEQ ID NO: 44)	No	No
10	16	SNESGWVWLP (SEQ ID NO: 110)	Yes	No
	17	EQSNSGWVWV (SEQ ID NO: 111)	Yes	No
	18	SRTESGWVWT (SEQ ID NO: 112)	Yes	No
	19	QRANSGWVWV (SEQ ID NO: 113)	Yes	No
	20	DYDNSGWVWH (SEQ ID NO: 114)	Yes	No
15	21	ETWGERDWFC (SEQ ID NO: 133)	Yes	Yes
	22	STAERLWFCG (SEQ ID NO: 135)	Yes	Yes
	23	YETAERSYFC (SEQ ID NO: 119)	Yes	Yes
	24	ADNAERGWFC (SEQ ID NO: 137)	Yes	Yes
	25	QNSSEREWFC (SEQ ID NO: 138)	Yes	Yes
20	26	STSERAWFCG (SEQ ID NO: 139)	Yes	Yes
	27	ASWSERGWFC (SEQ ID NO: 140)	Yes	Yes
	28	ELSSEREWFC (SEQ ID NO: 141)	Yes	Yes
	29	DMQGERGWFC (SEQ ID NO: 142)	Yes	Yes
	30	DMVYAYPPWS (SEQ ID NO: 155)	Yes	No
25	31	DEMVTVPYW (SEQ ID NO: 156)	Yes	Yes
	32	HTTNEQFFMC (SEQ ID NO: 434 )	Yes	Yes
	33	DTWLELESRY (SEQ ID NO: 435)	Yes	No

Ex. No.	Sequence	Specific Binding ?	Competitive Binding ?
34	DWQKTIPAYW (SEQ ID NO: 437)	Yes	Yes

### **EXAMPLES 35-73**

#### **G-CSF RADIOLIGAND BINDING ASSAYS**

5        The peptides of Table 2 were synthesized using standard techniques and were subsequently evaluated to determine their binding affinities to G-CSFR.

Streptavidin-coated scintillation proximity assay (SPA) beads (Amersham) were mixed with biotinylated anti-receptor immobilizing antibody (Ab179) followed by incubation with soluble G-CSFR harvest. Receptor-coated SPA beads were washed twice  
10       in PBS /0.1% BSA and distributed to wells of a white polystyrene 96-well microtiter plate (Packard). Serial dilutions of peptide or peptide mimetic were mixed with a constant amount of <sup>125</sup>I labeled G-CSF (10<sup>5</sup> cpm; 1290 Ci/mmol) in PBS/0.1% BSA, added to wells containing receptor-coated SPA beads, and incubated overnight at 4 °C. The binding of  
15       radiolabeled G-CSF to the receptor-coated SPA bead brings the isotope in close proximity to the scintillant, which allows the emitted radiation to stimulate the scintillant to emit light. Any unbound radiolabeled ligand is not in close enough proximity to the scintillant to allow such energy transfer and hence no signal is generated. The amount of <sup>125</sup>I labeled G-CSF that was bound at equilibrium was measured by counting the plate in a TopCount (Wallac) microtiter plate luminometer. The assay is conducted over a range of peptide  
20       concentrations and the results are graphed such that the y-axis represents the amount of bound <sup>125</sup>I labeled G-CSF and the x-axis represents the concentration of peptide or peptide mimetic. One can determine the concentration at which the peptide or peptide mimetic will reduce by 50% (IC<sub>50</sub>) the amount of <sup>125</sup>I labeled G-CSF bound to immobilized G-CSFR. The dissociation constant (K<sub>d</sub>) for the peptide should be similar to the measured  
25       IC<sub>50</sub> using the assay conditions described above.

The peptides along with their corresponding IC<sub>50</sub> values are shown in Table 2. IC<sub>50</sub> values are indicated symbolically by the symbols "-", "+", and "++". For examples, those peptides which showed IC<sub>50</sub> values in excess of 200 uM are indicated with a "-". Those peptides which gave IC<sub>50</sub> values of less than or equal to 200 uM are given a "+", while

those which gave  $IC_{50}$  values of 500 nM or less are indicated with a "++". Those peptides, which gave  $IC_{50}$  values at or near the cutoff point for a particular symbol, are indicated with a hybrid designator, e.g., "+/-". The peptides for which  $IC_{50}$  values were not determined are listed as "N.D.".

- 5           The results of these assays reveal important information about the structure-activity relationship for peptide and peptide mimetics of the invention to the G-CSF receptor.

Table 2

Ex. No.	Sequence	$IC_{50}$
35	$NH_2$ -EQSNSGWVWV-CONH <sub>2</sub> (SEQ ID NO: 111)	+
36	$NH_2$ -STAERLWFCG-CONH <sub>2</sub> (SEQ ID NO: 135)	-
37	$NH_2$ -STAERLWFCG-CONH <sub>2</sub> (SEQ ID NO: 135) $NH_2$ -STAERLWFCG-CONH <sub>2</sub> (SEQ ID NO: 135)	+
38	$NH_2$ -QNSEREWFC-CONH <sub>2</sub> (SEQ ID NO: 138)	-
39	$NH_2$ -QNSEREWFC-CONH <sub>2</sub> (SEQ ID NO: 138) $NH_2$ -QNSEREWFC-CONH <sub>2</sub> (SEQ ID NO: 138)	-
40	$NH_2$ -QNSEREWFCG-CONH <sub>2</sub> (SEQ ID NO: 149)	-
41	$NH_2$ -QNSEREWFCG-CONH <sub>2</sub> (SEQ ID NO: 149) $NH_2$ -QNSEREWFCG-CONH <sub>2</sub> (SEQ ID NO: 149)	-
42	Ac-ESGWVW-CONH <sub>2</sub> (SEQ ID NO: 470)	-
43	Ac-NSGWVW-CONH <sub>2</sub> (SEQ ID NO: 471)	-
44	Ac-SGWVW-CONH <sub>2</sub> (SEQ ID NO: 472)	-
45	$NH_2$ -EQSNSGWVWVGGGGC-CONH <sub>2</sub> (SEQ ID NO: 101)	+
46	$NH_2$ -EQSNSGWVWVGGGGC-CONH <sub>2</sub> (SEQ ID NO: 101) $NH_2$ -EQSNSGWVWVGGGGC-CONH <sub>2</sub> (SEQ ID NO: 101)	+
47	CESRLVECSRM (SEQ ID NO: 462)	+/-
48	LAHCLLRLEECAAG (SEQ ID NO: 460)	+/-
49	ALLMCESKLAECARAR (SEQ ID NO: 450)	+/-

5	50	DLWYLESKLEECARRANG (SEQ ID NO: 339) DLWYLESKLEECARRANG (SEQ ID NO: 339)	+
	51	DLWYLESKLEECARRCNG (SEQ ID NO: 340)	+
	52	DLWYLESKLEEAARRCNG (SEQ ID NO: 475) DLWYLESKLEEAARRCNG (SEQ ID NO: 475)	+
	53	LLDICELKLQECARRCN (SEQ ID NO: 208)	++
	54	GGGLLDICELKLQECARRCN (SEQ ID NO: 209)	++
10	55	GRTGGGLLDICELKLQECARRCN (SEQ ID NO: 210)	++
	56	LGIEGRTGGGLLDICELKLQECARRCN (SEQ ID NO: 211)	++
	57	LLDICELKLQECARRAN (SEQ ID NO: 343)	+
	58	LLDICELKLQEAARRCN (SEQ ID NO: 212)	+
	59	Biotin-LLDICELKLQECARRAN (SEQ ID NO: 343)	+
15	60	Biotin-KLLDICELKLQEAARRCN (SEQ ID NO: 213)	+
	61	LLDIAELKLQECARRCN (SEQ ID NO: 463)	+
	62	Biotin-KLLDIAELKLQECARRCN (SEQ ID NO: 464)	+
	63	Biotin-KGGGMLAERKAEERRWFNTHGRE (SEQ ID NO: 490)	+
	64	MLAERKAEERRWFNTHGRE (SEQ ID NO: 377) MLAERKAEERRWFNTHGREK (SEQ ID NO: 378)	+/-
20	65	CMLAERKAEERRWFNTHGRE (SEQ ID NO: 380) CMLAERKAEERRWFNTHGREK (SEQ ID NO: 381)	N.D.
	66	H <sub>2</sub> N-KSTGGGLTAERDAEKRRWLLTHGGE-COOH (SEQ ID NO: 491)	-
	67	CSTGGGLTAERDAEKRRWLLTHGGE (SEQ ID NO: 465) CSTGGGLTAERDAEKRRWLLTHGGE (SEQ ID NO: 465)	+
	68	LTAERDAEKRRWLLTHGGEGG (SEQ ID NO: 466) LTAERDAEKRRWLLTHGGEGGK (SEQ ID NO: 467)	-
	69	LTAERDAEKRRWLLTHGGEGGGGG (SEQ ID NO: 468) LTAERDAEKRRWLLTHGGEGGGGGK (SEQ ID NO: 469)	-
	70	YLELCQLRLEECARQFN (SEQ ID NO: 282)	+

71	CGCHVSPVQIKALC (SEQ ID NO: 198)	+
72	GCHVSPVQIKALC (SEQ ID NO: 199)	-
73	HELCETYADWLGCVEW (SEQ ID NO: 76)	N.D.

5

**EXAMPLES 74-81****CELL PROLIFERATION AND LUMINESCENCE ASSAYS**

The bioactivity of selected peptides of the invention was measured in cell-based assays. Murine NFS-60 cells proliferate in the presence of G-CSF in a dose dependent manner and were used in standard cell proliferation assays that are well known in the art.

10 Murine IL-3 dependent Ba/F3 cells were co-transfected with expression vectors encoding the full length human G-CSFR and a luciferase reporter gene controlled by the fos promoter. The Ba/F3 G-CSFR reporter cell line is not only dependent on the presence of G-CSF for proliferation, but also produces luciferase in response to the addition of G-CSF in a dose dependent manner. The parental, untransfected cell line does not respond to G-  
15 CSF or produce luciferase, but remains IL-3 dependent.

Reporter cell assays were performed on the above cell line using peptides of the invention. The cells were maintained in complete RPMI-1640 media containing 10% fetal calf serum, 2 mM L-glutamine, 1X antibiotic-antimycotic solution (Life Technologies), and 10% WEHI-3 conditioned media (source of murine IL-3). For reporter assays, cells  
20 were starved overnight in medium which lacks WEHI-3 to reduce luciferase expression to background levels. The cells were then washed twice in PBS, resuspended in media which lacks WEHI-3 conditioned media, and added to wells of a 96-well microtiter plate containing dilutions of peptide or G-CSF at  $5 \times 10^4$  cells/well. Plates were incubated for 2 hours at 37 °C in a humidified 5% CO<sub>2</sub> incubator and luciferase activity was measured by  
25 the addition of luciferin (LucLite - Packard Biosciences) to each well. The plates were read in a TopCount (Wallac) microtiter plate luminometer.

To measure the ability of selected peptides of the invention to block G-CSF mediated receptor activation, dilutions of peptide were combined with Ba/F3 G-CSFR reporter cells as described above. After a 30-minute incubation at 37 °C, G-CSF was

added to each well. The cells were incubated for 2 hours at 37 °C and the amount of luciferase produced was measured as described above.

The following seven peptides were tested for bioactivity:

- |    |        |   |
|----|--------|---|
| 5  | Ex. 74 | NH <sub>2</sub> -EQSNSGWVWV-CONH <sub>2</sub> (SEQ ID NO: 111); |
|    | Ex. 75 | NH <sub>2</sub> -STAERLWFCG-CONH <sub>2</sub> (SEQ ID NO: 135); |
|    | Ex. 76 | NH <sub>2</sub> -STAERLWFCG-CONH <sub>2</sub> (SEQ ID NO: 135); |
|    |        | NH <sub>2</sub> -STAERLWFCG-CONH <sub>2</sub> (SEQ ID NO: 135); |
| 10 | Ex. 77 | QLETCVLKLEECARRCN (SEQ ID NO: 315);                             |
|    | Ex. 78 | LLDICELKLQECARRCN (SEQ ID NO: 208);                             |
|    | Ex. 79 | PLFSCELKKQECARRCN (SEQ ID NO: 323); and                         |
|    | Ex. 80 | DLWYLESKLEECARRCN (SEQ ID NO: 338).                             |

- 15        Examples 74, 75, and 76 showed antagonist activity at high concentrations in cell-based assays using NFS-60 cells. The stability of Example 74 in cell culture medium was tested by overnight incubation in NFS-60-conditioned medium; no loss of activity was observed, indicating that the peptide is stable to degradation under these conditions.

- 20        Examples 77, 78, 79, and 80 showed cell proliferation activity when fused to the carboxy-terminus of the maltose binding protein (MBP). The MBP fusion protein of Example 78 in particular showed high affinity in a binding competition assay with <sup>125</sup>I-GCSF (IC<sub>50</sub> ~10 nM) and activity in a Ba/F3 G-CSFR cell proliferation assay (maximal activity at 100 nM). Parental Ba/F3 cells and Ba/F3 cells expressing the human thrombopoietin receptor did not proliferate in response to this fusion protein. Western blot
- 25        analysis of the fusion protein revealed both monomeric and dimeric species, however the G-CSFR preferentially binds the dimeric molecule. This is true for most of the MBP fusions tested. Presumably the fusion protein is dimerized through intermolecular disulfide bonds between cysteine residues present in the peptide sequence. Cleavage of
- 30        the peptide from the carboxy terminus of MBP using Factor Xa caused the peptide to lose its bioactivity while retaining its binding activity.

The Ba/F3 G-CSFR reporter cell line was used to measure the potency of:

- Ex. 81        LLDICELKLQECARRCN (SEQ ID NO: 208)

and other possible G-CSF receptor antagonists.

Ligand mediated G-CSF receptor activation in these cells results in the expression of luciferase, providing a detectable biological signal. Ba/F3 G-CSFR reporter cells responded to the addition of G-CSF in a dose dependent manner (Figure 2). The addition of increasing concentrations of peptide from Example 81 inhibit this G-CSF response, indicating that the peptide is a G-CSFR antagonist (Figure 3).

## **EXAMPLE 82**

### **CHARACTERIZATION OF THE DIMER FORM OF AF15846**

The peptide AF15846, i.e., LLDICELKLQECARRCN (SEQ ID NO: 208), was under study as a G-CSF antagonist for chemoprotection against chemotherapy-induced neutropenia. The peptide monomer contains three Cys residues with a mass of 2020.4 (average). This peptide is not active as a monomer but must be oxidized, putatively to a dimer form, for activity.

#### **Monomer vs. dimer forms of AF15846:**

AF15846 that had been oxidized in 50 mM Tris, pH 8.0 for 48 hours was diluted with PBS, then injected onto a Superdex peptide gel filtration column equilibrated in PBS at 0.75 mL/min. The results of this chromatography indicated that most of the peptide was in dimer form, with small amounts of monomer remaining (not shown). In contrast, AF15846 that had been stored in acid and then diluted with PBS directly prior to injection onto the peptide column eluted predominantly as a monomer. Some dimerization apparently occurred either during storage or during the short period the peptide was at neutral pH prior to and during size exclusion chromatography. Oxidized peptide also eluted much later from a cation exchange column run in salt gradients at low pH, consistent with dimer formation (not shown).

#### **Reverse phase HPLC assay for oxidation of AF15846:**

AF15846 was oxidized by incubation in 50 mM Tris, pH 8.0, for 16 to 48 hours. Reverse phase HPLC methods using a Vydac 25 cm C-18 column and 0.1% TFA/acetonitrile buffers were developed to separate the oxidized dimer from unoxidized

monomer, and to separate several different dimerized peptide structures. While both high pH reverse phase and cation exchange chromatography were also investigated, low pH reverse phase separation on a 25 cm column provided the best separation of the many oxidized forms of the peptide (not shown). The dimer species elute from the column with earlier retention times than do the monomer species. Samples of oxidized AF15846 were re-reduced with DTT to confirm the elution order. One additional piece of evidence for the formation of intermolecular dimers comes from the fact that when oxidation was carried out at low (0.25 mg/mL) concentrations of peptide, the reaction apparently did not go to completion.

#### **Oxidation of AF15846 under various conditions:**

AF15846 was incubated for 48 hours in 50 mM Tris, pH 8, 20% DMSO in water, 20 mM potassium phosphate, pH 3, or 0.1% TFA at room temperature. Aliquots of each sample were taken at various time points. Oxidation of the monomer peptide in Tris resulted in the presence of one major plus one minor oxidized species after several hours. In contrast, oxidation of the peptide in 20% DMSO in water resulted in a complex mixture of oxidized species, even after the 48 hour incubation. Some oxidation of the peptide was observed even at acidic pH, although to a much lesser extent than that observed with either Tris or DMSO as the oxidant.

#### **Activity of oxidized AF15846 fractions:**

Several fractions containing oxidized AF15846 resulting from treatment under the conditions described above were collected subjected to testing in two assays: an  $^{125}\text{I}$ -G-CSF competition binding assay and an ELISA format competitive G-CSF receptor-binding assay. In both cases fractions corresponding to the predominant Tris-oxidized species exhibited the highest activity. The activity of selected fractions in the  $^{125}\text{I}$ -G-CSF competition binding assay is shown in Figure 4. While species corresponding to the monomer peptide were inactive, matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) confirmed that the active, Tris-oxidized species was a peptide dimer.



**Determination of the disulfide structure of the active oxidized form of AF15846:**

It was hypothesized that the active form of AF15846 would contain one intrachain disulfide per peptide monomer and one interchain peptide dimer. The three possibilities for this type of structure are shown below

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10

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20

$H_3N^+$ -LLDICE LKLQECARRCN-COO<sup>-</sup> (SEQ ID NO: 208)

$H_3N^+$ -LLDICE LKLQECARRCN-COO<sup>-</sup> (SEQ ID NO: 208);

$H_3N^+$ -LLDICE LKLQECARRCN-COO<sup>-</sup> (SEQ ID NO: 208)

$H_3N^+$ -LLDICE LKLQECARRCN-COO<sup>-</sup> (SEQ ID NO: 208); and

$H_3N^+$ -LLDICE LKLQECARRCN-COO<sup>-</sup> (SEQ ID NO: 208)

$H_3N^+$ -LLDICE LKLQECARRCN-COO<sup>-</sup> (SEQ ID NO: 208).

25 To determine if one of these structures was present in the active form of AF15846, aliquots of Tris-oxidized AF15846 (not HPLC purified) were digested with trypsin and subjected to reverse phase HPLC. Trypsin digestion was carried out using an immobilized enzyme column from Perseptive Biosystems. Digestion was carried out in 25 mM Tris, pH 8, 5 mM CaCl<sub>2</sub>. Fractions were eluted from the column directly into 0.1% TFA to lower the

30 pH and minimize disulfide scrambling. The resulting tryptic fragments were separated by reverse phase HPLC and analyzed by MALDI mass spectrometry and Edman sequencing. In addition, an aliquot of the digest was analyzed by electrospray liquid chromatography/mass spectrometry (LC/MS). MALDI MS and sequencing of the tryptic peptides indicated the presence of peptides corresponding to disulfide bonds between Cys-

35 5 and Cys-5, as well as between Cys12 and Cys-12. This finding indicated that there were two interchain disulfide bonds between peptide monomers. This result was confirmed by the LC/MS data (Figure 5), which identified peptides identical to those found by MALDI MS. The tryptic peptides are labeled, beginning with the first residue, i.e., Lys, as follows: T1 = residues 1-8; T2 = residues 9-14; T1,2 = residues 1-14; T2,3 = residues 9-15; and "+" indicates a disulfide linkage between peptides. However, an additional minor species was

40 evidently present, as a peptide corresponding to a disulfide bond between Cys-5 and Cys-

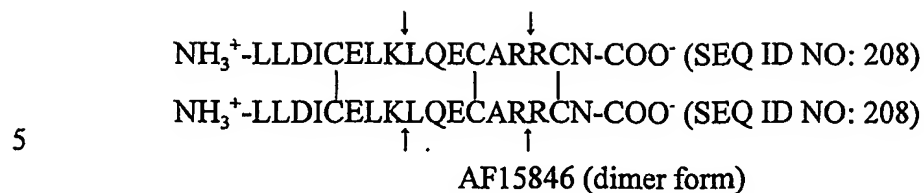
12, which could be either an intrachain or an interchain disulfide, was also seen, albeit at a lower level.

To confirm that the active species contained at least two interchain disulfides, an aliquot of the HPLC-purified, Tris-oxidized AF15846 shown to be active in competition  
5 assays was also digested with trypsin. The profile of the purified material was compared to that of the unfractionated Tris oxidation product (Figure 6, same labeling as in Figure 5). The HPLC profile indicates that the purified material is lacking a peptide corresponding to a Cys-5 to Cys-12 disulfide-linked fragment. This indicated that the active species contains two interchain disulfide bonds. However, the oxidation state of the  
10 remaining Cys-16 in each monomer was not determined.

The oxidized peptide was also reacted with N-ethylmaleimide (NEM) at 37 °C for 1 hour in 100 mM ammonium acetate, pH 4.1 to see if any free Cys residues remained in the molecule. If this were the case, treatment with the alkylating reagent would result in a shift of the HPLC retention time. Upon incubation with NEM, no such shift was seen  
15 (Figure 7). In contrast, when the oxidized peptide was incubated with the disulfide specific reducing agent TCEP, also in ammonium acetate, a shift to a later retention time, consistent with reduced peptide, was found. The reduced peptide was modified with NEM to produce a peptide that eluted even later than the reduced form. These data indicate that all six Cys residues in the AF15846 active dimer are involved in disulfide bonds. Since  
20 previous results showed that Cys-5 is linked to Cys-5 and Cys-12 is linked to Cys-12, it seems apparent that the remaining two Cys residues at position 16 of the monomer are also involved in an interchain disulfide bond.

To obtain further information about the disulfide bond structure in active AF15846, the peptide was digested with Lys-C in 50 mM Tris pH 7.0/30% acetonitrile. The profile of  
25 this digest is shown in Figure 8. Four major peaks are seen. The first peak corresponds to a dimer of residues 9-17, as indicated by the MALDI MS spectrum of this fraction. See Figures 9A and 9B. However, it is not possible to tell with this technique if all four Cys residues are involved in disulfide formation. The last peak contains a dimer of residues 1-8. The remaining two peaks represent intact peptide (22 min) and an artifact peak. This  
30 second digest clearly indicates that the peptide dimerizes into a parallel structure.

This three parallel interchain disulfide structure, indicated below, is different than that originally predicted. Note that the arrows represent sites of cleavage by trypsin.



Incubation of the oxidized peptide at 37 °C at higher pH apparently resulted disulfide scrambling and/or degradation of the peptide as control peptide fractions incubated at pH 6.0 or pH 7.5 in parallel with NEM-treated fractions exhibited complex HPLC patterns after incubation. It was necessary to drop to pH 4.1 to obtain clean profiles upon NEM treatment.

#### A bioassay for determining activity of G-CSF antagonists:

A biosassay was used to measure the potency of AF15846 and other possible G-CSF receptor antagonists. This bioassay utilizes a Ba/F3 cell line containing the rhGCSF receptor and a c-fos promoter/luciferase gene construct (Ba/F3/rhGCSF-R/pFos-lcf). Competent binding of a ligand to the receptor results in expression of luciferase as the biological readout. Addition of AF15846 to the assay results in the dose-response curve shifting to higher concentrations, indicating that the peptide is inhibiting the binding of G-CSF to the expressed receptor (Figures 10A and 10B). Conversely, the inclusion of various levels of peptide in the assay causes an increase in the amount of G-CSF required to produce a signal, also indicating that the peptide inhibits G-CSF binding (Figure 11).

CLAIMS

What is claimed is:

1. A compound comprising a peptide chain approximately 10 to 40 amino acids in length that binds to G-CSFR and contains a sequence of amino acids of formula (I)

5 (I)  $CX_1X_2X_3X_4X_5X_6X_7X_8C$  (SEQ ID NO: 1)

wherein each amino acid is indicated by standard one-letter abbreviation, and wherein  $X_1$  is A, N, S, F, D, G, L, T, E, V, P, Q, H, M or K;  $X_2$  is M, G, R, H, D, I, V, A, S, E, N, F, Y, P, C, W or T;  $X_3$  is E, V, W, F, M, A, N, S, L, T, Y, G or P;  $X_4$  is V, I, G, Q, W, M, T, Y, L, P, D, C, E or A;  $X_5$  is M, E, W, L, P, N, I, T, V, F, Y, Q, S, R, W, G, H or D;  $X_6$  is  
 10 H, A, W, Y, V, F, Q, M, N, E, S, D, P or G;  $X_7$  is M, F, Y, V, N, L, H, D, S, W, G, Q, C or T; and  $X_8$  is C, Y, R, I, K, W, L, E, M, H, A, T, F, D, P, G or Q.

2. The compound of claim 1, wherein  $X_1$  is D or P,  $X_2$  is D or P,  $X_3$  is E or W,  $X_4$  is V, I or Y,  $X_5$  is M or L,  $X_6$  is W, Y or F,  $X_7$  is M, Y or D, and  $X_8$  is C or M.

15

3. The compound of claim 1, wherein the sequence of amino acids is selected from the group consisting of:

CAGEVMHMCC (SEQ ID NO: 8);  
 CNREIEAMCC (SEQ ID NO: 9);  
 20 CADEVMHFCC (SEQ ID NO: 10);  
 CNREIMWMCC (SEQ ID NO: 11);  
 CSHEVWWYCC (SEQ ID NO: 12);  
 CSREVLYYCC (SEQ ID NO: 13);  
 CFIEGPWVCC (SEQ ID NO: 14);  
 25 CFVEGNWYCC (SEQ ID NO: 15);  
 CAAEVMVNCC (SEQ ID NO: 16);  
 CSDEVIFYCC (SEQ ID NO: 17);  
 CDREIMWFCC (SEQ ID NO: 18);  
 CAHEVMWMCC (SEQ ID NO: 19);  
 30 CGSEVTFMCC (SEQ ID NO: 20);  
 CLEEIMWLCC (SEQ ID NO: 21);

CAREVLAMCC (SEQ ID NO: 22);  
CSVEVMQMCC (SEQ ID NO: 23);  
CTNVQLMHYC (SEQ ID NO: 24);  
CDVWQLFDRC (SEQ ID NO: 25);  
5 CSFVQLNSIC (SEQ ID NO: 26);  
CDYWQWFDKC (SEQ ID NO: 27);  
CESFWVELWC (SEQ ID NO: 28);  
CVPWMFYDLC (SEQ ID NO: 29);  
CDPWMFYDLC (SEQ ID NO: 30);  
10 CDPWVLFDEC (SEQ ID NO: 31);  
CDHWTYFDMC (SEQ ID NO: 32);  
CVVWTLYDKC (SEQ ID NO: 33);  
CPDWYQSYMC (SEQ ID NO: 34);  
CPDWYSYYMC (SEQ ID NO: 35);  
15 CPEWYTDVMC (SEQ ID NO: 36);  
CPDWYLDYMC (SEQ ID NO: 37);  
CPEWYLDYMC (SEQ ID NO: 38);  
CPDWYLPYMC (SEQ ID NO: 39);  
CPEWYLPYMC (SEQ ID NO: 40);  
20 CQDWWVELWC (SEQ ID NO: 41);  
CPDWYLPWMC (SEQ ID NO: 42);  
CACMLRVVHC (SEQ ID NO: 43);  
CQRAGYMLAC (SEQ ID NO: 44);  
CHANPVWGEC (SEQ ID NO: 45);  
25 CFWSDWGQTC (SEQ ID NO: 46);  
CPHWTSYYMC (SEQ ID NO: 47);  
CETLCGACFC (SEQ ID NO: 48);  
CATTINDTLC (SEQ ID NO: 49);  
CLNYPHPVFC (SEQ ID NO: 50);  
30 CMDGEMAVDC (SEQ ID NO: 51);  
CNMGWMSWPC (SEQ ID NO: 52);  
CETYADWLGC (SEQ ID NO: 53);

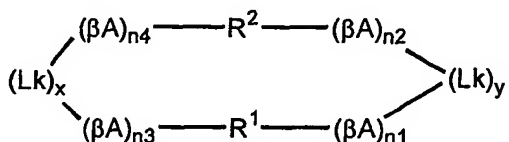
- 5 CDPWMFFDMC (SEQ ID NO: 54);  
CDPWIWYDLC (SEQ ID NO: 55);  
CDPWIMYDRC (SEQ ID NO: 56);  
CDPWVFFDIC (SEQ ID NO: 57);  
CDPWTYYDLC (SEQ ID NO: 58);  
CDPWIFYDRC (SEQ ID NO: 59);  
CDPWLFYDLC (SEQ ID NO: 60);  
CDPWVWYDLC (SEQ ID NO: 61);  
CDPWIFFDRC (SEQ ID NO: 62);  
10 CDPWMFFDQC (SEQ ID NO: 63);  
CDPWLWYDRC (SEQ ID NO: 64);  
CDVWVWYDQC (SEQ ID NO: 65);  
CDPWIYYDLC (SEQ ID NO: 66);  
CVPWTLFDLC (SEQ ID NO: 67);  
15 CPAWYLEYMC (SEQ ID NO: 68);  
CPDWYLEYMC (SEQ ID NO: 69);  
CKYWQWFDKC (SEQ ID NO: 70); and  
CDHWMWYDKC (SEQ ID NO: 71).
- 20 4. The compound of claim 1, wherein the sequence of amino acids is selected from the group consisting of:
- GCNREIEAMCCG (SEQ ID NO: 72);  
GCPEWYTDVMCG (SEQ ID NO: 73);  
NWYCMDGEMAVDCEAT (SEQ ID NO: 74);  
25 WQSCNMGWMSWPCYFV (SEQ ID NO: 75);  
HELCETYADWLGCVEW (SEQ ID NO: 76);  
PCDPWMFFDMCERW (SEQ ID NO: 77);  
LRGCDPWIWYDLCPAV (SEQ ID NO: 78);  
GYLCDPWIXYDRCLGF (SEQ ID NO: 79);  
30 RFACDPWVFFDICGYW (SEQ ID NO: 80);  
GYWCDPWTYYDLCLTA (SEQ ID NO: 81);  
MWTCDPWIFYDRCFLN (SEQ ID NO: 82);

GSSCDPWLFYDLCLLD (SEQ ID NO: 83);  
 GGGCDPWVWYDLCWCD (SEQ ID NO: 84);  
 YTSCDPWIFFDRCMSV (SEQ ID NO: 85);  
 DPYCDPWMFFDQCAYL (SEQ ID NO: 86);  
 5 REFCDPWLWYDRCL (SEQ ID NO: 87);  
 NTGCDVWVWYDQCFAM (SEQ ID NO: 88);  
 LVFCDPWIIYYDLCMDT (SEQ ID NO: 89);  
 GCSFVQLNSICG (SEQ ID NO: 90);  
 GCPAWYLEYMCG (SEQ ID NO: 91);  
 10 GCPDWYLEYMCG (SEQ ID NO: 92);  
 GCKYWQWFDKCG (SEQ ID NO: 93); and  
 GCDHWMWYDKCG (SEQ ID NO: 94).

5. The compound of claim 1, comprising a dimer having the structure of formula

15 (VIII)

(VIII)



20 wherein  $R^1$  and  $R^2$  are independently selected from the sequences of amino acids of formula (I);  $\beta A$  is a  $\beta$ -alanine residue;  $n1$ ,  $n2$ ,  $n3$ ,  $n4$ ,  $x$  and  $y$  are independently zero or one with the proviso that the sum of  $x$  and  $y$  is either one or two; and  $Lk$  is a terminal linking moiety selected from the group consisting of a disulfide bond, a carbonyl moiety, a  $C_{1-12}$  linking moiety optionally terminated with one or two  $-NH-$  linkages and optionally  
 25 substituted at one or more available carbon atoms with a lower alkyl substituent, a lysine residue or a lysine amide.

6. A compound comprising a peptide chain approximately 9 to 40 amino acids in length that binds to G-CSFR and contains a sequence of amino acids of formula (II)

30 (II)  $X^1_1 X^1_2 X^1_3 SGWVWX^1_4$  (SEQ ID NO: 2)

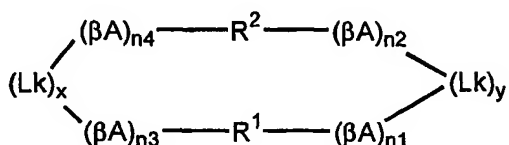
wherein each amino acid is indicated by the standard one-letter abbreviation, and wherein  $X^1_1$  is S, Q, R, L or Y;  $X^1_2$  is N, S, T, A or D;  $X^1_3$  is E, D or N; and  $X^1_4$  is L, V, T, P or H.

7. The compound of claim 6, wherein the sequence of amino acids is selected from the group consisting of:

SNESGWVWL (SEQ ID NO: 95);  
 QSNSGWVWV (SEQ ID NO: 96);  
 5 RTESGWVWT (SEQ ID NO: 97);  
 RANSGWVWV (SEQ ID NO: 98);  
 YDNSGWVWH (SEQ ID NO: 99); and  
 LSDSGWVWVP (SEQ ID NO: 100).

10 8. The compound of claim 6, comprising a dimer having the structure of formula (VIII)

(VIII)



15

wherein  $R^1$  and  $R^2$  are independently selected from the sequences of amino acids of formula (II);  $\beta A$  is a  $\beta$ -alanine residue;  $n1$ ,  $n2$ ,  $n3$ ,  $n4$ ,  $x$  and  $y$  are independently zero or one with the proviso that the sum of  $x$  and  $y$  is either one or two; and  $Lk$  is a terminal linking moiety selected from the group consisting of a disulfide bond, a carbonyl moiety, a  $C_{1-12}$  linking moiety optionally terminated with one or two  $-NH-$  linkages and optionally substituted at one or more available carbon atoms with a lower alkyl substituent, a lysine residue or a lysine amide.

9. The compound of claim 8, wherein the dimer is:  
 25  $NH_2$ -EQNSGWVWVGGGGC- $CONH_2$  (SEQ ID NO: 101)  
 $NH_2$ -EQNSGWVWVGGGGC- $CONH_2$  (SEQ ID NO: 101);

10. A compound comprising a peptide chain approximately 6 to 40 amino acids in length that binds to G-CSFR and contains a sequence of amino acids of formula (III)

(III)  $ERX^I_1X^I_2X^I_3C$  (SEQ ID NO: 3)

30



wherein each amino acid is indicated by standard one-letter abbreviation, and wherein  $X^{\text{II}}_1$  is D, L, S, G, E, A, K or Y;  $X^{\text{II}}_2$  is W, Y, F, L or V; and  $X^{\text{II}}_3$  is F, G, M or L.

11. The compound of claim 10, wherein  $X^{\text{II}}_1$  is D or L,  $X^{\text{II}}_2$  is W, and  $X^{\text{II}}_3$  is F.

5

12. The compound of claim 10, wherein the sequence of amino acids is selected from the group consisting of:

ERDWFC (SEQ ID NO: 120);

ERDWGC (SEQ ID NO: 121);

10 ERLWFC (SEQ ID NO: 122);

ERSYFC (SEQ ID NO: 123);

ERGWFC (SEQ ID NO: 124);

EREWFC (SEQ ID NO: 125);

ERAWFC (SEQ ID NO: 126);

15 ERLYFC (SEQ ID NO: 127);

ERYFMC (SEQ ID NO: 128);

ERLFLC (SEQ ID NO: 129);

ERALMC (SEQ ID NO: 130);

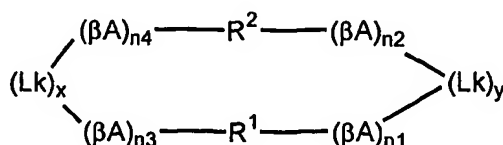
ERDVMC (SEQ ID NO: 131); and

20 ERKWFC (SEQ ID NO: 132).

13. The compound of claim 10, comprising a dimer having the structure of formula (VIII)

25

(VIII)



30

wherein  $R^1$  and  $R^2$  are independently selected from the sequences of amino acids of formula (III);  $\beta A$  is a  $\beta$ -alanine residue;  $n1$ ,  $n2$ ,  $n3$ ,  $n4$ ,  $x$  and  $y$  are independently zero or one with the proviso that the sum of  $x$  and  $y$  is either one or two; and  $Lk$  is a terminal linking moiety selected from the group consisting of a disulfide bond, a carbonyl moiety, a  $C_{1-12}$  linking moiety optionally terminated with one or two  $-NH-$  linkages and optionally

substituted at one or more available carbon atoms with a lower alkyl substituent, a lysine residue or a lysine amide.

14. The compound of claim 13, selected from the group consisting of:

5  $\text{NH}_2\text{-STAERLWFCG-CONH}_2$  (SEQ ID NO: 135)

$\text{NH}_2\text{-STAERLWFCG-CONH}_2$  (SEQ ID NO: 135);

10  $\text{NH}_2\text{-QNSEREWFC-CONH}_2$  (SEQ ID NO: 138)

$\text{NH}_2\text{-QNSEREWFC-CONH}_2$  (SEQ ID NO: 138); and

15  $\text{NH}_2\text{-QNSEREWFCG-CONH}_2$  (SEQ ID NO: 149)

$\text{NH}_2\text{-QNSEREWFCG-CONH}_2$  (SEQ ID NO: 149).

15. A compound comprising a peptide chain approximately 9 to 40 amino acids in length that binds to G-CSFR and contains a sequence of amino acids of formula (IV)

(IV)  $\text{X}^{\text{III}}_1\text{M}^{\text{III}}\text{VYX}^{\text{III}}_2\text{X}^{\text{III}}_3\text{P}^{\text{III}}_4\text{W}$  (SEQ ID NO: 4)

20 wherein each amino acid is indicated by standard one-letter abbreviation, and wherein  $\text{X}^{\text{III}}_1$  is D or E;  $\text{X}^{\text{III}}_2$  is A or T;  $\text{X}^{\text{III}}_3$  is Y or V; and  $\text{X}^{\text{III}}_4$  is P or Y.

16. The compound of claim 15, wherein the sequence of amino acids is selected from the group consisting of:

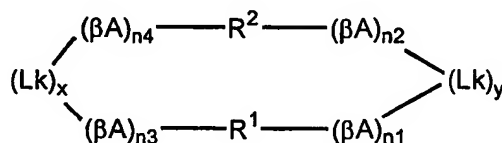
25  $\text{DMVYAYPPW}$  (SEQ ID NO: 153); and

$\text{EMVYTVPYW}$  (SEQ ID NO: 154).

17. The compound of claim 15, comprising a dimer having the structure of formula (VIII)

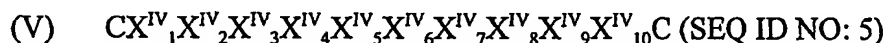
30

(VIII)



wherein R<sup>1</sup> and R<sup>2</sup> are independently selected from the sequences of amino acids of formula (IV); βA is a β-alanine residue; n<sub>1</sub>, n<sub>2</sub>, n<sub>3</sub>, n<sub>4</sub>, x and y are independently zero or one with the proviso that the sum of x and y is either one or two; and Lk is a terminal linking moiety selected from the group consisting of a disulfide bond, a carbonyl moiety, a C<sub>1-12</sub> linking moiety optionally terminated with one or two -NH- linkages and optionally substituted at one or more available carbon atoms with a lower alkyl substituent, a lysine residue or a lysine amide.

18. A compound comprising a peptide chain approximately 12 to 40 amino acids in length that binds to G-CSFR and contains a sequence of amino acids of formula (V)



wherein each amino acid is indicated by standard one-letter abbreviation, and wherein X<sup>IV</sup><sub>1</sub> is E, G, P, N, R, T, W, S, L, H, A, Q or Y; X<sup>IV</sup><sub>2</sub> is S, T, E, A, D, G, W, P, L, N, V, Y, R or M; X<sup>IV</sup><sub>3</sub> is R, Y, V, Q, E, T, L, P, S, K, M, A or W; X<sup>IV</sup><sub>4</sub> is L, M, G, F, W, R, S, V, P, A, D, C or T; X<sup>IV</sup><sub>5</sub> is V, T, A, R, S, L, W, C, I, E, P, H, F, D or Q; X<sup>IV</sup><sub>6</sub> is E, Y, G, T, Q, M, S, N, A or P; X<sup>IV</sup><sub>7</sub> is C, V, D, G, L, W, E, V, I, S, M or A; X<sup>IV</sup><sub>8</sub> is S, Y, A, W, P, V, L, Q, G, K, F, I, E or D; X<sup>IV</sup><sub>9</sub> is R, W, M, D, H, V, G, A, Q, L, S, E or Y; X<sup>IV</sup><sub>10</sub> is M, L, I, S, V, P, W, F, T, Y, R, or Q.

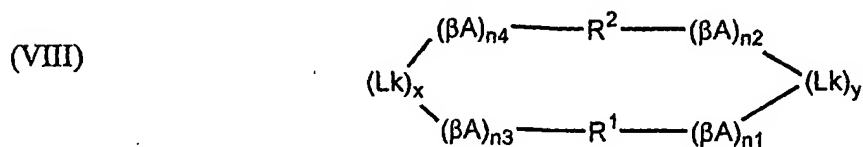
19. The compound of claim 18, wherein X<sup>IV</sup><sub>1</sub> is E, X<sup>IV</sup><sub>2</sub> is S or A, X<sup>IV</sup><sub>3</sub> is R, X<sup>IV</sup><sub>4</sub> is L, X<sup>IV</sup><sub>5</sub> is V or S, X<sup>IV</sup><sub>6</sub> is E, X<sup>IV</sup><sub>7</sub> is C, X<sup>IV</sup><sub>8</sub> is S, X<sup>IV</sup><sub>9</sub> is R, and X<sup>IV</sup><sub>10</sub> is L.

20. The compound of claim 18, wherein the sequence of amino acids is selected from the group consisting of:

25 CESRLVECSRMC (SEQ ID NO: 157);  
 CETYMTYVYWLC (SEQ ID NO: 158);  
 CGERLAECARLC (SEQ ID NO: 159);  
 CESRLRECSMLC (SEQ ID NO: 160);  
 CEARLSECSRIC (SEQ ID NO: 161);  
 30 CPARLLECSRMC (SEQ ID NO: 162);  
 CESVGVDWWSC (SEQ ID NO: 163);  
 CEDRLVEGPWVC (SEQ ID NO: 164);

CNDQFRTCVDVC (SEQ ID NO: 165);  
 CRGEWWELYHPC (SEQ ID NO: 166);  
 CEDTRTGWAWSC (SEQ ID NO: 167);  
 CTWLSSGELVWC (SEQ ID NO: 168);  
 5 CWPPVCEVSGIC (SEQ ID NO: 169);  
 CSLSPIQLQHLC (SEQ ID NO: 170);  
 CLARLEECSRFC (SEQ ID NO: 171);  
 CHNSSPMVGVTC (SEQ ID NO: 172);  
 CHVSPVQIKALC (SEQ ID NO: 173);  
 10 CAAPATSWFQYC (SEQ ID NO: 174);  
 CASKLHECSLRC (SEQ ID NO: 175);  
 CEPMDSNGIVQC (SEQ ID NO: 176);  
 CQYASAADEQRC (SEQ ID NO: 177);  
 CEYWDEPSLSWC (SEQ ID NO: 178);  
 15 CERECFQMLERC (SEQ ID NO: 179);  
 CGMSTDELDEIC (SEQ ID NO: 180);  
 CYVSPSTGLYSC (SEQ ID NO: 181);  
 CEARLVECSRLC (SEQ ID NO: 182);  
 CESRLSECSRMC (SEQ ID NO: 183);  
 20 CELKLQECARRC (SEQ ID NO: 184);  
 CELKLQEAARRC (SEQ ID NO: 185); and  
 CLERLEECSRFC (SEQ ID NO: 186).

21. The compound of claim 18, comprising a dimer having the structure of  
 25 formula (VIII)



30 wherein  $\text{R}^1$  and  $\text{R}^2$  are independently selected from the sequences of amino acids of  
 formula (V);  $\beta\text{A}$  is a  $\beta$ -alanine residue;  $n_1$ ,  $n_2$ ,  $n_3$ ,  $n_4$ ,  $x$  and  $y$  are independently zero or  
 one with the proviso that the sum of  $x$  and  $y$  is either one or two; and  $\text{Lk}$  is a terminal

linking moiety selected from the group consisting of a disulfide bond, a carbonyl moiety, a C<sub>1-12</sub> linking moiety optionally terminated with one or two -NH- linkages and optionally substituted at one or more available carbon atoms with a lower alkyl substituent, a lysine residue or a lysine amide.

5

22. The compound of claim 21, having the structure:

NH<sub>3</sub><sup>+</sup>-LLDICE<sup>|</sup>KLQECARRCN-COO<sup>-</sup> (SEQ ID NO: 208)

NH<sub>3</sub><sup>+</sup>-LLDICE<sup>|</sup>KLQECARRCN-COO<sup>-</sup> (SEQ ID NO: 208).

10

23. A compound comprising a peptide chain approximately 9 to 40 amino acids in length that binds to G-CSFR and contains a sequence of amino acids of formula (VI)

(VI) X<sup>V</sup><sub>1</sub>X<sup>V</sup><sub>2</sub>X<sup>V</sup><sub>3</sub>X<sup>V</sup><sub>4</sub>X<sup>V</sup><sub>5</sub>X<sup>V</sup><sub>6</sub>CX<sup>V</sup><sub>7</sub>X<sup>V</sup><sub>8</sub> (SEQ ID NO: 6)

wherein each amino acid is indicated by standard one-letter abbreviation, and wherein X<sup>V</sup><sub>1</sub> is E, C, Q, V, or Y; X<sup>V</sup><sub>2</sub> is E, A, L, M, S, W, or Q; X<sup>V</sup><sub>3</sub> is K, R or T; X<sup>V</sup><sub>4</sub> is L, A, or V; X<sup>V</sup><sub>5</sub> is R, A, M, H, E, V, L, G, D, Q, or S; X<sup>V</sup><sub>6</sub> is E or V; X<sup>V</sup><sub>7</sub> is A or G; X<sup>V</sup><sub>8</sub> is R, H, G or L.

15

24. The compound of claim 23, wherein X<sup>V</sup><sub>1</sub> is E, X<sup>V</sup><sub>2</sub> is A or L, X<sup>V</sup><sub>3</sub> is K or R, X<sup>V</sup><sub>4</sub> is L, X<sup>V</sup><sub>6</sub> is E, X<sup>V</sup><sub>7</sub> is A, and X<sup>V</sup><sub>8</sub> is R:

20

25. The compound of claim 23, wherein the sequence of amino acids is selected from the group consisting of:

EEKLRECAR (SEQ ID NO: 214);

EARLAECAR (SEQ ID NO: 215);

25 CMKLMECAR (SEQ ID NO: 216);

ELRLRECAH (SEQ ID NO: 217);

EAKLHECAR (SEQ ID NO: 218);

ELKLAECAR (SEQ ID NO: 219);

EARLEECAR (SEQ ID NO: 220);

30 EAKLRECAR (SEQ ID NO: 221);

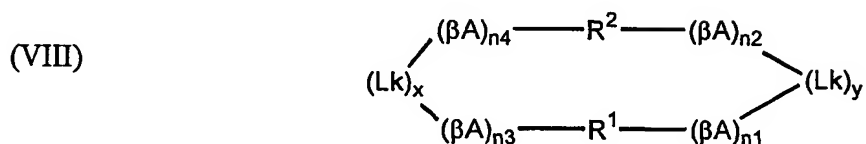
ELRLAECAR (SEQ ID NO: 222);

ESRLAECAR (SEQ ID NO: 223);

EAKLVECAR (SEQ ID NO: 224);  
ESRLRECAR (SEQ ID NO: 225);  
EAKLAECAR (SEQ ID NO: 226);  
QWRLEECAR (SEQ ID NO: 227);  
5 QLRLEECAR (SEQ ID NO: 228);  
ELRLEECAR (SEQ ID NO: 229);  
EAKLLECAR (SEQ ID NO: 230);  
EARAGVCAG (SEQ ID NO: 231);  
EAKAGVCAG (SEQ ID NO: 232);  
10 VARLEECAR (SEQ ID NO: 233);  
ELKLDECAR (SEQ ID NO: 234);  
EWRLQECAR (SEQ ID NO: 235);  
EAKLSECAR (SEQ ID NO: 236);  
EARLSECAR (SEQ ID NO: 237);  
15 ELKLLECAR (SEQ ID NO: 238);  
ELRLQECGR (SEQ ID NO: 239);  
EQKLAECAR (SEQ ID NO: 240);  
ELRLQECAR (SEQ ID NO: 241);  
ELKLEECAR (SEQ ID NO: 242);  
20 ESRLEECAR (SEQ ID NO: 243);  
EATVQECAR (SEQ ID NO: 244);  
ELKLQECAR (SEQ ID NO: 245);  
YSRLEECGR (SEQ ID NO: 246);  
ELRLRECAL (SEQ ID NO: 247);  
25 EARLLECAR (SEQ ID NO: 248);  
ESRLLECAR (SEQ ID NO: 249);  
VLKLEECAR (SEQ ID NO: 250);  
ESKLAECAR (SEQ ID NO: 251);  
ESKLRECAR (SEQ ID NO: 252);  
30 EYKLGECA (SEQ ID NO: 253);  
ESRLQECAR (SEQ ID NO: 254);  
QARLAECAR (SEQ ID NO: 255);

ELKKQECAR (SEQ ID NO: 256);  
 ESRLSECAR (SEQ ID NO: 257);  
 EARLEECGR (SEQ ID NO: 258);  
 ESRLAECGR (SEQ ID NO: 259);  
 5 EWRLEECAR (SEQ ID NO: 260);  
 EARLSECGR (SEQ ID NO: 261);  
 AARLAECAR (SEQ ID NO: 262);  
 EWKLAECAR (SEQ ID NO: 263);  
 ESKLEECAR (SEQ ID NO: 264);  
 10 DVKLAECAR (SEQ ID NO: 265);  
 ELQLEECAR (SEQ ID NO: 266); and  
 EYKLASCAR (SEQ ID NO: 267).

26. The compound of claim 23, comprising a dimer having the structure of  
 15 formula (VIII)



20 wherein  $R^1$  and  $R^2$  are independently selected from the sequences of amino acids of formula (V);  $\beta A$  is a  $\beta$ -alanine residue;  $n_1$ ,  $n_2$ ,  $n_3$ ,  $n_4$ ,  $x$  and  $y$  are independently zero or one with the proviso that the sum of  $x$  and  $y$  is either one or two; and  $Lk$  is a terminal linking moiety selected from the group consisting of a disulfide bond, a carbonyl moiety, a  $C_{1-12}$  linking moiety optionally terminated with one or two  $-NH-$  linkages and optionally  
 25 substituted at one or more available carbon atoms with a lower alkyl substituent, a lysine residue or a lysine amide.

27. The compound of claim 26, selected from the group consisting of:

30 [H]-DLWYLESKLEECARRANG-[NH<sub>2</sub>] (SEQ ID NO: 339)  
 [H]-DLWYLESKLEECARRANG-[NH<sub>2</sub>] (SEQ ID NO: 339);

[H]-DLWYLESKLEECARRCNG -[NH<sub>2</sub>] (SEQ ID NO: 340); and

[H]-LLDICEKLQECARRAN-[OH] (SEQ ID NO: 343).

28. A compound comprising a peptide chain approximately 10 to 40 amino acids in length that binds to G-CSFR and contains a sequence of amino acids of formula (VII)

(VII)  $X^{VI}_1X^{VI}_2X^{VI}_3X^{VI}_4X^{VI}_5EX^{VI}_6X^{VI}_7X^{VI}_8X^{VI}_9$  (SEQ ID NO: 7)

wherein each amino acid is indicated by standard one-letter abbreviation, and wherein  $X^{VI}_1$  is A, E or G;  $X^{VI}_2$  is E, H or D;  $X^{VI}_3$  is R or G;  $X^{VI}_4$  is K, Y, M, N, Q, R, D, I, S or E;  $X^{VI}_5$  is A, S or P;  $X^{VI}_6$  is E, D, T, Q, K or A;  $X^{VI}_7$  is R, W, K, L, S, A or Q;  $X^{VI}_8$  is R or E; and  $X^{VI}_9$  is W, G, or R.

29. The compound of claim 28, wherein the sequence of amino acids is selected from the group consisting of:

AERKAEERRW (SEQ ID NO: 344);

AERYAEEREG (SEQ ID NO: 345);

AERMAEERRW (SEQ ID NO: 346);

AERKAEERRR (SEQ ID NO: 347);

AHRNAEERRW (SEQ ID NO: 348);

AERKSEDWRW (SEQ ID NO: 349);

AERKAEERKRR (SEQ ID NO: 350);

AERQAETRRW (SEQ ID NO: 351);

AERNAEERRW (SEQ ID NO: 352);

AERQAEERRW (SEQ ID NO: 353);

AERRAEERRW (SEQ ID NO: 354);

AERDAEQRRW (SEQ ID NO: 355);

AERIAEERRW (SEQ ID NO: 356);

AERSAEERRW (SEQ ID NO: 357);

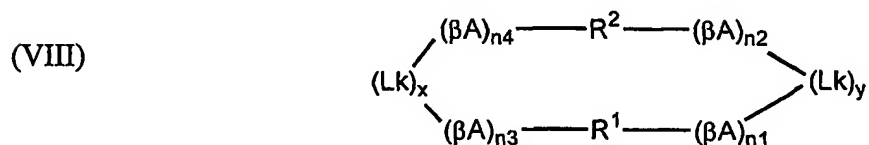
AERKAEELRW (SEQ ID NO: 358);

AERKAEESRW (SEQ ID NO: 359);



EERKAEERRW (SEQ ID NO: 360);  
 ADGKAEERRW (SEQ ID NO: 361);  
 ADGKAEELRW (SEQ ID NO: 362);  
 ADGMPEERRW (SEQ ID NO: 363);  
 5 ADGEAEKRRW (SEQ ID NO: 364);  
 ADGNAEERRW (SEQ ID NO: 365);  
 ADGEAEKARW (SEQ ID NO: 366);  
 AEGEAEKARW (SEQ ID NO: 367);  
 GERKAEERRW (SEQ ID NO: 368);  
 10 AEREAERRW (SEQ ID NO: 369);  
 ADGEAEARRW (SEQ ID NO: 370);  
 ADGRAEEARW (SEQ ID NO: 371);  
 AEGRAEEARW (SEQ ID NO: 372);  
 AEREA EKARW (SEQ ID NO: 373);  
 15 AERKAEQRW (SEQ ID NO: 374);  
 AERDAEKRRW (SEQ ID NO: 375); and  
 AEREA EKLRW (SEQ ID NO: 376).

20 30. The compound of claim 28, comprising a dimer having the structure of  
 formula (VIII)



25 wherein  $R^1$  and  $R^2$  are independently selected from the sequences of amino acids of  
 formula (VI);  $\beta A$  is a  $\beta$ -alanine residue;  $n1$ ,  $n2$ ,  $n3$ ,  $n4$ ,  $x$  and  $y$  are independently zero or  
 one with the proviso that the sum of  $x$  and  $y$  is either one or two; and  $Lk$  is a terminal  
 linking moiety selected from the group consisting of a disulfide bond, a carbonyl moiety, a  
 $C_{1-12}$  linking moiety optionally terminated with one or two  $-NH-$  linkages and optionally  
 30 substituted at one or more available carbon atoms with a lower alkyl substituent, a lysine  
 residue or a lysine amide.

31. The compound of claim 30, wherein the dimer is selected from the group consisting of:

MLAERKAEERRWFNTHGRE (SEQ ID NO: 377)

5 MLAERKAEERRWFNTHGRE-K(NH<sub>2</sub>) (SEQ ID NO: 378) and

CMLAERKAEERRWFNTHGRE (SEQ ID NO: 380)

CMLAERKAEERRWFNTHGRE-K (SEQ ID NO: 381).

10

32. The compound of any one of claims 1, 6, 10, 15, 18, 23 or 28, containing a disulfide bond.

33. The compound of any one of claims 1, 6, 10, 15, 18, 23 or 28, wherein the N-terminus of the peptide is coupled to a polyethylene glycol molecule.

15

34. The compound of any one of claims 1, 6, 10, 15, 18, 23 or 28, wherein the N-terminus of the peptide is acetylated.

35. The compound of any one of claims 1, 6, 10, 15, 18, 23 or 28, wherein the C-terminus of the peptide is amidated.

20

36. A pharmaceutical composition comprising a therapeutically effective amount of the compound of any one of claims 1, 6, 10, 15, 18, 23 or 28, in combination with a pharmaceutically acceptable carrier.

25

37. A method for treating a patient who would benefit from administration of a G-CSF modulator, comprising administering to the patient a therapeutically effective amount of the compound of any one of claims 1, 6, 10, 15, 18, 23 or 28.

30

38. The method of claim 37, wherein the G-CSF modulator is an agonist for the G-CSFR.

39. The method of claim 38, wherein the patient suffers from a depressed neutrophil count.

40. The method of claim 39, wherein the depressed neutrophil count is caused a  
5 condition selected from the group consisting of chemotherapy-induced neutropenia, AIDS-induced neutropenia and community-acquired pneumonia-induced neutropenia.

41. A compound comprising a peptide chain approximately 6 to 40 amino acids in  
length that binds to G-CSF and contains a sequence of amino acids selected from the  
10 group consisting of:

CTWTDLESVY (SEQ ID NO: 433);  
HTTNEQFFMC (SEQ ID NO: 434);  
DTWLELESRY (SEQ ID NO: 435);  
HNSSPMVGVT (SEQ ID NO: 436);  
15 DWQKTIPAYW (SEQ ID NO: 437);  
RWGREGLVAALL (SEQ ID NO: 438);  
WSGTRVWRCVVT (SEQ ID NO: 439);  
MSLLSYLRS (SEQ ID NO: 440);  
LDLLAI (SEQ ID NO: 441);  
20 RIYGVK (SEQ ID NO: 442);  
MIWHMFMSLLF (SEQ ID NO: 443);  
FFWASWMHLLW (SEQ ID NO: 444);  
FDDCWREREQFLFQAL (SEQ ID NO: 445);  
CGRASECFRLLEM (SEQ ID NO: 446);  
25 RECFQMLER (SEQ ID NO: 447);  
CSIRWDFVPGYGLC (SEQ ID NO: 448);  
WMQCWDSLRLCYDM (SEQ ID NO: 449);  
ALLMCESKLAECARAR (SEQ ID NO: 450);  
LAHCKKRKEECAAG (SEQ ID NO: 451);  
30 SIDGVYLRTSRT (SEQ ID NO: 452);  
SIDGVYLRTSRTRY (SEQ ID NO: 453);  
VWRLRGSTLRGLRD (SEQ ID NO: 454);

DRGGGTGVGYWWESY (SEQ ID NO: 455);  
VWGTVTGTWLEY (SEQ ID NO: 456);  
LMWVSAY (SEQ ID NO: 457);  
RASDEYGALVRFCTNL (SEQ ID NO: 458);  
5 NYWCDSNWVCEIA (SEQ ID NO: 459);  
LAHCLLRLEECAAG (SEQ ID NO: 460);  
LALCLARLRECAGG (SEQ ID NO: 461);  
CESRLVECSRM (SEQ ID NO: 462);  
LLDIAELKLQECARRCN (SEQ ID NO: 463);  
10 KLLDIAELKLQECCARRCN (SEQ ID NO: 464);  
CSTGGGLTAERDAEKRRWLLTHGGE (SEQ ID NO: 465)  
LTAERDAEKRRWLLTHGGEGG (SEQ ID NO: 466);  
LTAERDAEKRRWLLTHGGEGGK (SEQ ID NO: 467);  
LTAERDAEKRRWLLTHGGEGGGGG (SEQ ID NO: 468);  
15 LTAERDAEKRRWLLTHGGEGGGGGK (SEQ ID NO: 469);  
ESGWVW (SEQ ID NO: 470);  
NSGWVW (SEQ ID NO: 471);  
SGWVW (SEQ ID NO: 472);  
PLGKCEATCREMARYFN (SEQ ID NO: 473);  
20 SLQRCEYKLASVRGLCN (SEQ ID NO: 474)  
DLWYLESKLEEAARRCNG (SEQ ID NO: 475);  
PYMGTRSRKLLRQQ (SEQ ID NO: 476);  
RNAGERRWFKTQGWY (SEQ ID NO: 477);  
MLAERNADRRWFNTHGRD (SEQ ID NO: 478);  
25 MMADGRLRNSVGLILWCD (SEQ ID NO: 479);  
MLADGRLRNVVG (SEQ ID NO: 480);  
LLADVRRRNGVGLLRMGRD (SEQ ID NO: 481);  
MLADGRLRNFGG (SEQ ID NO: 482);  
TYMTYVYWLC (SEQ ID NO: 483); (CORE 158)  
30 RFGERWGL (SEQ ID NO: 484);  
HWLWWGWNF (SEQ ID NO: 485);  
RECFQMLERC (SEQ ID NO: 486);

ILAHRNAKERRWFQKHGR (SEQ ID NO: 487); and  
CSTGGGLTAERDAEKRRWLLTHGGEK (SEQ ID NO: 489).

Figure 1-1

CAGEVMHMCC (SEQ ID NO: 8)  
CNREIEAMCC (SEQ ID NO: 9)  
CADEVMHFCC (SEQ ID NO: 10)  
CNREIMWMCC (SEQ ID NO: 11)  
CSHEVWWYCC (SEQ ID NO: 12)  
CSREVLYYCC (SEQ ID NO: 13)  
CFIEGPWVCC (SEQ ID NO: 14)  
CFVEGNWYCC (SEQ ID NO: 15)  
CAAHEVMVNCC (SEQ ID NO: 16)  
CSDEVIFYCC (SEQ ID NO: 17)  
CDREIMWFCC (SEQ ID NO: 18)  
CAHEVMWMCC (SEQ ID NO: 19)  
CGSEVTFMCC (SEQ ID NO: 20)  
CLEEIMWLCC (SEQ ID NO: 21)  
CAREVLAMCC (SEQ ID NO: 22)  
CSVEVMQMCC (SEQ ID NO: 23)  
CTNVQLMHYC (SEQ ID NO: 24)  
CDVWQLFDRC (SEQ ID NO: 25)  
CSFVQLNSIC (SEQ ID NO: 26)  
CDYWQWFDKC (SEQ ID NO: 27)  
CESFWVELWC (SEQ ID NO: 28)  
CVPWMFYDLC (SEQ ID NO: 29)  
CDPWMFYDLC (SEQ ID NO: 30)  
CDPWVLFDEC (SEQ ID NO: 31)  
CDHWTYFDMC (SEQ ID NO: 32)  
CVVWTLYDKC (SEQ ID NO: 33)  
CPDWYQSYMC (SEQ ID NO: 34)  
CPDWYSYYMC (SEQ ID NO: 35)  
CPEWYTDVMC (SEQ ID NO: 36)  
CPDWYLDYMC (SEQ ID NO: 37)  
CPEWYLDYMC (SEQ ID NO: 38)  
CPDWYLPYMC (SEQ ID NO: 39)  
CPEWYLPYMC (SEQ ID NO: 40)  
CQDWWVELWC (SEQ ID NO: 41)  
CPDWYLPWMC (SEQ ID NO: 42)  
CACMLRVVHC (SEQ ID NO: 43)  
CQRAGYMLAC (SEQ ID NO: 44)  
CHANPVWGEC (SEQ ID NO: 45)  
CFWSDWGQTC (SEQ ID NO: 46)  
CPHWTSYYMC (SEQ ID NO: 47)  
CETLCGACFC (SEQ ID NO: 48)  
CATTINDTLC (SEQ ID NO: 49)  
CLNYPHPVFC (SEQ ID NO: 50)

Figure 1-2

CMDGEMAVDC (SEQ ID NO: 51)  
CNMGWMSWPC (SEQ ID NO: 52)  
CETYADWLGC (SEQ ID NO: 53)  
CDPWMFFDMC (SEQ ID NO: 54)  
CDPWIWYDLC (SEQ ID NO: 55)  
CDPWIMYDRC (SEQ ID NO: 56)  
CDPWVFFDIC (SEQ ID NO: 57)  
CDPWTTYDLC (SEQ ID NO: 58)  
CDPWIFYDRC (SEQ ID NO: 59)  
CDPWLFYDLC (SEQ ID NO: 60)  
CDPWVWYDLC (SEQ ID NO: 61)  
CDPWIFFDRC (SEQ ID NO: 62)  
CDPWMFFDQC (SEQ ID NO: 63)  
CDPWLWYDRC (SEQ ID NO: 64)  
CDVWVWYDQC (SEQ ID NO: 65)  
CDPWIYYDLC (SEQ ID NO: 66)  
CVPWTLFDLC (SEQ ID NO: 67)  
CPAWYLEYMC (SEQ ID NO: 68)  
CPDWYLEYMC (SEQ ID NO: 69)  
CKYWQWFDKC (SEQ ID NO: 70)  
CDHWMWYDKC (SEQ ID NO: 71)  
GCNREIEAMCCG (SEQ ID NO: 72)  
GCPEWYTDVMCG (SEQ ID NO: 73)  
NWYCMDGEMAVDCEAT (SEQ ID NO: 74)  
WQSCNMGWMSWPCYFV (SEQ ID NO: 75)  
HELCETYADWLGCVEW (SEQ ID NO: 76)  
PCDPWMFFDMCERW (SEQ ID NO: 77)  
LRGCDPWIWYDLCPAV (SEQ ID NO: 78)  
GYLCDPWIFYDRCLGF (SEQ ID NO: 79)  
RFACDPWVFFDICGYW (SEQ ID NO: 80)  
GYWCDPWTTYDLCLTA (SEQ ID NO: 81)  
MWTCDPWIFYDRCLN (SEQ ID NO: 82)  
GSSCDPWLFYDLCLLD (SEQ ID NO: 83)  
GGGCDPWVWYDLCWCD (SEQ ID NO: 84)  
YTSCDPWIFFDRCMSV (SEQ ID NO: 85)  
DPYCDPWMFFDQCAYL (SEQ ID NO: 86)  
REFCDPWLWYDRCL (SEQ ID NO: 87)  
NTGCDVWVWYDQCFAM (SEQ ID NO: 88)  
LVFCDPWIYYDLCDT (SEQ ID NO: 89)  
GCSFVQLNSICG (SEQ ID NO: 90)  
GCPAWYLEYMCG (SEQ ID NO: 91)  
GCPDWYLEYMCG (SEQ ID NO: 92)  
GCKYWQWFDKCG (SEQ ID NO: 93)  
GCDHWMWYDKCG (SEQ ID NO: 94)  
SNESGWVWL (SEQ ID NO: 95)

Figure 1-3

QNSNSGWVWV (SEQ ID NO: 96)  
RTESGWVWT (SEQ ID NO: 97)  
RANSGWVWV (SEQ ID NO: 98)  
YDNSGWVWH (SEQ ID NO: 99)  
LSDSGWVVVP (SEQ ID NO: 100)  
EQSNSGWVWVGGGGC (SEQ ID NO: 101)  
CEQSNSGWVWV (SEQ ID NO: 102)  
EQSNSGWVWVGGGGCKKK (SEQ ID NO: 103)  
EQSNSGWVWVGKKKC (SEQ ID NO: 104)  
EQSNSGWVWVGKKK (SEQ ID NO: 105)  
KKKEQSNSGWVWV (SEQ ID NO: 106)  
EQSNSGWVWVGKKKSKKK (SEQ ID NO: 107)  
EQSNSGWVWVGCKKK (SEQ ID NO: 108)  
EQSNSGWVWVGGGGGCKKK (SEQ ID NO: 109)  
SNESGWVWLP (SEQ ID NO: 110)  
EQSNSGWVWV (SEQ ID NO: 111)  
SRTEGWVWT (SEQ ID NO: 112)  
QRANSGWVWV (SEQ ID NO: 113)  
DYDNSGWVWH (SEQ ID NO: 114)  
EQSNSGWVWVGKKKK (SEQ ID NO: 115)  
EQSNSGWVWVGGGGSKKK (SEQ ID NO: 116)  
EQSNSGWVWVGGGGS (SEQ ID NO: 117)  
EQSNSGWVWVGGGGSEQSNSGWVWVGGGGS (SEQ ID NO: 118)  
RYQSFELSDSGWVVVPVARH (SEQ ID NO: 119)  
ERDWFC (SEQ ID NO: 120)  
ERDWGC (SEQ ID NO: 121)  
ERLWFC (SEQ ID NO: 122)  
ERSYFC (SEQ ID NO: 123)  
ERGWFC (SEQ ID NO: 124)  
EREWFC (SEQ ID NO: 125)  
ERAWFC (SEQ ID NO: 126)  
ERLYFC (SEQ ID NO: 127)  
ERYFMC (SEQ ID NO: 128)  
ERLFLC (SEQ ID NO: 129)  
ERALMC (SEQ ID NO: 130)  
ERDVMC (SEQ ID NO: 131)  
ERKWFC (SEQ ID NO: 132)  
ETWGERDWFC (SEQ ID NO: 133)  
ETWGERDWGC (SEQ ID NO: 134)  
STAERLWFCG (SEQ ID NO: 135)  
YETAERSYFC (SEQ ID NO: 136)  
ADNAERGWFC (SEQ ID NO: 137)  
QNSSEREWFC (SEQ ID NO: 138)  
STSERAWFCG (SEQ ID NO: 139)  
ASWSERGWFC (SEQ ID NO: 140)



## Figure 1-4

ELSSEREWFC (SEQ ID NO: 141)  
DMQGERGWFC (SEQ ID NO: 142)  
SSSERA WFCG (SEQ ID NO: 143)  
GNMRERLYFC (SEQ ID NO: 144)  
QPNRERYFMC (SEQ ID NO: 145)  
SVTRERLFLC (SEQ ID NO: 146)  
IPLSERALMCSSWNC (SEQ ID NO: 147)  
WARSERDVMCLSYVC (SEQ ID NO: 148)  
QNSEREWFCG (SEQ ID NO: 149)  
QNSEREWFCGGGS (SEQ ID NO: 150)  
NLEEALAQERLWFCRSGNC (SEQ ID NO: 151)  
NLESYEMEERKWFCCKMFSC (SEQ ID NO: 152)  
DMVYAYPPW (SEQ ID NO: 153)  
EMVYTVPYW (SEQ ID NO: 154)  
DMVYAYPPWS (SEQ ID NO: 155)  
DEMVYTVPYW (SEQ ID NO: 156)  
CESRLVECSRMC (SEQ ID NO: 157)  
CETYMTYVYWLC (SEQ ID NO: 158)  
CGERLAECARLC (SEQ ID NO: 159)  
CESRLRECSMLC (SEQ ID NO: 160)  
CEARLSECSRIC (SEQ ID NO: 161)  
CPARLLECSRMC (SEQ ID NO: 162)  
CESVGVGDWWSC (SEQ ID NO: 163)  
CEDRLVEGPWVC (SEQ ID NO: 164)  
CNDQFRTCVDVC (SEQ ID NO: 165)  
CRGEWWELYHPC (SEQ ID NO: 166)  
CEDTRTGAWWSC (SEQ ID NO: 167)  
CTWLSSGELVWC (SEQ ID NO: 168)  
CWPPVCEVSGIC (SEQ ID NO: 169)  
CSLSPIQLQHLC (SEQ ID NO: 170)  
CLARLEECSRFC (SEQ ID NO: 171)  
CHNSSPMVGVTC (SEQ ID NO: 172)  
CHVSPVQIKALC (SEQ ID NO: 173)  
CAAPATSWFQYC (SEQ ID NO: 174)  
CASKLHECSLRC (SEQ ID NO: 175)  
CEPMDSNGIVQC (SEQ ID NO: 176)  
CQYASAADEQRC (SEQ ID NO: 177)  
CEYWDEPSLSWC (SEQ ID NO: 178)  
CERECFQMLERC (SEQ ID NO: 179)  
CGMSTDELDEIC (SEQ ID NO: 180)  
CYVSPSTGLYSC (SEQ ID NO: 181)  
CEARLVECSRLC (SEQ ID NO: 182)  
CESRLSECSRMC (SEQ ID NO: 183)  
CELKLQECARRC (SEQ ID NO: 184)  
CELKLQEAARRC (SEQ ID NO: 185)

## Figure 1-5

CLERLEECRFC (SEQ ID NO: 186)  
GGCESRLVECSRMC (SEQ ID NO: 187)  
GGCETYMTYVYWLC (SEQ ID NO: 188)  
EWLCESVGVGDWWSC (SEQ ID NO: 189)  
YHPCEDRLVEGPWVCCRS (SEQ ID NO: 190)  
WLLCNDQFRTCVDVCDNV (SEQ ID NO: 191)  
IAECRGEWWELYHPCCLAA (SEQ ID NO: 192)  
TWYCEDTRTGAWWSCLEL (SEQ ID NO: 193)  
QLDCTWLSSGELVWCSDW (SEQ ID NO: 194)  
QFDCTWLSSGELVWCSDW (SEQ ID NO: 195)  
CWPPVCEVSGICS (SEQ ID NO: 196)  
CGCSLSPIQLQHLC (SEQ ID NO: 197)  
CGCHVSPVQIKALC (SEQ ID NO: 198)  
GCHVSPVQIKALC (SEQ ID NO: 199)  
GTSCAAPATSWFQYCVLP (SEQ ID NO: 200)  
RMDCASKLHECSLRCA YA (SEQ ID NO: 201)  
GVVCEPMDSNGIVQCSMR (SEQ ID NO: 202)  
IDVCQYASAADEQRCLRI (SEQ ID NO: 203)  
NVLCEYWDEPSLSWCLSS (SEQ ID NO: 204)  
CQCERECFQMLERC (SEQ ID NO: 205)  
FCSCGMSTDELDEICAIW (SEQ ID NO: 206)  
EEVCYVSPSTGLYSCYDQ (SEQ ID NO: 207)  
LLDICELKLQECARRCN (SEQ ID NO: 208)  
GGGLLDICELKLQECARRCN (SEQ ID NO: 209)  
GRTGGGLLDICELKLQECARRCN (SEQ ID NO: 210)  
LGIEGRTGGGLLDICELKLQECARRCN (SEQ ID NO: 211)  
LLDICELKLQEAARRCN (SEQ ID NO: 212)  
KLLDICELKLQEAARRCN (SEQ ID NO: 213)  
EEKLRECAR (SEQ ID NO: 214)  
EARLAECAR (SEQ ID NO: 215)  
CMKLMECAR (SEQ ID NO: 216)  
ELRLRECAH (SEQ ID NO: 217)  
EAKLHECAR (SEQ ID NO: 218)  
ELKLAECAR (SEQ ID NO: 219)  
EARLEECAR (SEQ ID NO: 220)  
EAKLRECAR (SEQ ID NO: 221)  
ELRLAECAR (SEQ ID NO: 222)  
ESRLAECAR (SEQ ID NO: 223)  
EAKLVECAR (SEQ ID NO: 224)  
ESRLRECAR (SEQ ID NO: 225)  
EAKLAECAR (SEQ ID NO: 226)  
QWRLEECAR (SEQ ID NO: 227)  
QLRLEECAR (SEQ ID NO: 228)  
ELRLEECAR (SEQ ID NO: 229)  
EAKLLECAR (SEQ ID NO: 230)

Figure 1-6

EARAGVCAG (SEQ ID NO: 231)  
EAKAGVCAG (SEQ ID NO: 232)  
VARLEECAR (SEQ ID NO: 233)  
ELKLDECAR (SEQ ID NO: 234)  
EWRLQECAR (SEQ ID NO: 235)  
EAKLSECAR (SEQ ID NO: 236)  
EARLSECAR (SEQ ID NO: 237)  
ELKLLECAR (SEQ ID NO: 238)  
ELRLQECGR (SEQ ID NO: 239)  
EQKLAECAR (SEQ ID NO: 240)  
ELRLQECAR (SEQ ID NO: 241)  
ELKLEECAR (SEQ ID NO: 242)  
ESRLEECAR (SEQ ID NO: 243)  
EATVQECAR (SEQ ID NO: 244)  
ELKLQECAR (SEQ ID NO: 245)  
YSRLEECGR (SEQ ID NO: 246)  
ELRLRECAL (SEQ ID NO: 247)  
EARLLECAR (SEQ ID NO: 248)  
ESRLLECAR (SEQ ID NO: 249)  
VLKLEECAR (SEQ ID NO: 250)  
ESKLAECAR (SEQ ID NO: 251)  
ESKLRECAR (SEQ ID NO: 252)  
EYKLGECAR (SEQ ID NO: 253)  
ESRLQECAR (SEQ ID NO: 254)  
QARLAECAR (SEQ ID NO: 255)  
ELKKQECAR (SEQ ID NO: 256)  
ESRLSECAR (SEQ ID NO: 257)  
EARLEECGR (SEQ ID NO: 258)  
ESRLAECGR (SEQ ID NO: 259)  
EWRLEECAR (SEQ ID NO: 260)  
EARLSECGR (SEQ ID NO: 261)  
AARLAECAR (SEQ ID NO: 262)  
EWKLAECAR (SEQ ID NO: 263)  
ESKLEECAR (SEQ ID NO: 264)  
DVKLAECAR (SEQ ID NO: 265)  
ELQLEECAR (SEQ ID NO: 266)  
EYKLASCAR (SEQ ID NO: 267)  
RLSICEEKLRECARGC (SEQ ID NO: 268)  
PLTTCEARLAECARQL (SEQ ID NO: 269)  
LALCMKLMECARRY (SEQ ID NO: 270)  
ELVMCELRLRECAHRA (SEQ ID NO: 271)  
PLARCEAKLHECARQL (SEQ ID NO: 272)  
LLSVCELKLAECARSK (SEQ ID NO: 273)  
RLEWCEARLEECARRC (SEQ ID NO: 274)  
RLRVVEAKLRECARGR (SEQ ID NO: 275)

## Figure 1-7

CVAHLELRLAECARQI (SEQ ID NO: 276)  
HLARCESRLAECARQL (SEQ ID NO: 277)  
RLALLEAKLVECARRL (SEQ ID NO: 278)  
DLFSLESRLRECARRV (SEQ ID NO: 279)  
AVPVLEAKLAECARRF (SEQ ID NO: 280)  
YLQQLQWRLEECARGM (SEQ ID NO: 281)  
YLELCQLRLEECARQFN (SEQ ID NO: 282)  
ELHICELRLEECARGR (SEQ ID NO: 283)  
RVARCELRLAECARKS (SEQ ID NO: 284)  
YLEVLESRLAECARWK (SEQ ID NO: 285)  
EAKLLECARAR (SEQ ID NO: 286)  
ELSLCEARAGVCAGSVTK (SEQ ID NO: 287)  
ELSLCEAKAGVCAGSVTK (SEQ ID NO: 288)  
ALWQCVARLEECARSR (SEQ ID NO: 289)  
CLKSCELKLDECARRM (SEQ ID NO: 290)  
ALQTCEWRLQECARSR (SEQ ID NO: 291)  
YISQCEAKLAECARLY (SEQ ID NO: 292)  
ELSSCEAKLSECARRW (SEQ ID NO: 293)  
ELSSCEARLSECARRW (SEQ ID NO: 294)  
QLLQCELKLLEECARQG (SEQ ID NO: 295)  
ELLRCEARLAECARGC (SEQ ID NO: 296)  
QLRQCELRLQECGRHGN (SEQ ID NO: 297)  
PLTSCEQKLAECARRF (SEQ ID NO: 298)  
LLGMCELRLQECARAK (SEQ ID NO: 299)  
ELSRCELKLEECARGM (SEQ ID NO: 300)  
DCRPCEsrLEECARRL (SEQ ID NO: 301)  
RLSVCEARLEECARQL (SEQ ID NO: 302)  
PLKMCEATVQECARLI (SEQ ID NO: 303)  
LLLFCEARLSECARHV (SEQ ID NO: 304)  
SLSMCEARLAECARLL (SEQ ID NO: 305)  
PLFSCSELKLQECARRCN (SEQ ID NO: 306)  
SLERCYSRLEECGRRI (SEQ ID NO: 307)  
PLTSCSELRLRECALRSN (SEQ ID NO: 308)  
KLAACELKLAECARRW (SEQ ID NO: 309)  
KLAACELRLAECARRW (SEQ ID NO: 310)  
ALTRCELRLAECARKI (SEQ ID NO: 311)  
LLQQCELKLAECARSI (SEQ ID NO: 312)  
QLWQCEARLLEECARRS (SEQ ID NO: 313)  
RLRLCESRLLEECARSL (SEQ ID NO: 314)  
QLETCVLKLEECARRCN (SEQ ID NO: 315)  
ALSQCELRLAECARSVTK (SEQ ID NO: 316)  
ELKLAECARRS (SEQ ID NO: 317)  
ALSRCESKLAECARRQ (SEQ ID NO: 318)  
LMSTCESKLRECARSL (SEQ ID NO: 319)  
SLQRCEYKLGECARSL (SEQ ID NO: 320)

Figure 1-8

RLELLESRLQECARQLN (SEQ ID NO: 321)  
QMEWCQARLAECARCCN (SEQ ID NO: 322)  
PLFSCCLKQECARRCN (SEQ ID NO: 323)  
LLDKCESRLSECARRL (SEQ ID NO: 324)  
LLARCEARLEECGRQC (SEQ ID NO: 325)  
DLLYCESRLAECGRM (SEQ ID NO: 326)  
ALQMCEWRLEECARRL (SEQ ID NO: 327)  
LLTMCEARLSECGRRL (SEQ ID NO: 328)  
ALWRCESRLAECARRS (SEQ ID NO: 329)  
LLATCAARLAECARQL (SEQ ID NO: 330)  
LQTCEWKLAECARSN (SEQ ID NO: 331)  
PLRSCSKLEECARQL (SEQ ID NO: 332)  
CLRALDVKLAECARHL (SEQ ID NO: 333)  
RLKTLELQLEECARRS (SEQ ID NO: 334)  
KLRDVELKLAECARRS (SEQ ID NO: 335)  
SLQRCEYKLASCARSL (SEQ ID NO: 336)  
RLARCELRLAECARKS (SEQ ID NO: 337)  
DLWYLESKLEECARRCN (SEQ ID NO: 338)  
DLWYLESKLEECARRANG (SEQ ID NO: 339)  
DLWYLESKLEECARRCNG (SEQ ID NO: 340)  
KQRELELKLAECARRS (SEQ ID NO: 341)  
QMQEWCARLAECARCCN (SEQ ID NO: 342)  
LLDICELKLQECARRAN (SEQ ID NO: 343)  
AERKAEERRW (SEQ ID NO: 344)  
AERYAEEREG (SEQ ID NO: 345)  
AERMAEERRW (SEQ ID NO: 346)  
AERKAEERRR (SEQ ID NO: 347)  
AHRNAEERRW (SEQ ID NO: 348)  
AERKSEDWRW (SEQ ID NO: 349)  
AERKAEKRR (SEQ ID NO: 350)  
AERQAETRRW (SEQ ID NO: 351)  
AERNAEERRW (SEQ ID NO: 352)  
AERQAEERRW (SEQ ID NO: 353)  
AERRAEERRW (SEQ ID NO: 354)  
AERDAEQRRW (SEQ ID NO: 355)  
AERIAEERRW (SEQ ID NO: 356)  
AERSAEERRW (SEQ ID NO: 357)  
AERKAEELRW (SEQ ID NO: 358)  
AERKAEESRW (SEQ ID NO: 359)  
EERKAEERRW (SEQ ID NO: 360)  
ADGKAEERRW (SEQ ID NO: 361)  
ADGKAEELRW (SEQ ID NO: 362)  
ADGMPEERRW (SEQ ID NO: 363)  
ADGEAEKRRW (SEQ ID NO: 364)  
ADGNAEERRW (SEQ ID NO: 365)

Figure 1-9

ADGEAEKARW (SEQ ID NO: 366)  
AEGEAEKARW (SEQ ID NO: 367)  
GERKAEERRW (SEQ ID NO: 368)  
AEREAERRW (SEQ ID NO: 369)  
ADGEAEARRW (SEQ ID NO: 370)  
ADGRAEEARW (SEQ ID NO: 371)  
AEGRAEEARW (SEQ ID NO: 372)  
AEREAEKARW (SEQ ID NO: 373)  
AERKAEQQRW (SEQ ID NO: 374)  
AERDAEKRRW (SEQ ID NO: 375)  
AEREAEKLRW (SEQ ID NO: 376)  
MLAERKAEERRWFNTHGRE (SEQ ID NO: 377)  
MLAERKAEERRWFNTHGREK (SEQ ID NO: 378)  
GGGMLAERKAEERRWFNTHGRE (SEQ ID NO: 379)  
CMLAERKAEERRWFNTHGRE (SEQ ID NO: 380)  
CMLAERKAEERRWFNTHGREK (SEQ ID NO: 381)  
MLAERYAEEREGFNMQWRE (SEQ ID NO: 382)  
MLAERMAEERRWFRRMG (SEQ ID NO: 383)  
IVAERKAEERRRLNTEGHE (SEQ ID NO: 384)  
ILAHRNAEERRWFQKHGR (SEQ ID NO: 385)  
MLAERKSEDWRWLKTHGRD (SEQ ID NO: 386)  
MLAERKAEERRLKTQGRE (SEQ ID NO: 387)  
ILAERQAETRRWMRNAGSVTK (SEQ ID NO: 388)  
MLAERNAEERRWLKRQCG (SEQ ID NO: 389)  
MLAERQAEERRWLKMHGGE (SEQ ID NO: 390)  
MLAERRAEERRWLKTQGGD (SEQ ID NO: 391)  
MLAERQAEERRWLKTQGRD (SEQ ID NO: 392)  
MLAERKAEERRWFKTHGRE (SEQ ID NO: 393)  
MLAERKAEERRWFNNQGRE (SEQ ID NO: 394)  
MPAERDAEQRRWLKTHGRE (SEQ ID NO: 395)  
ILAERIAEERRWLKTQGR (SEQ ID NO: 396)  
MLAERKAEERRWLQTHGRE (SEQ ID NO: 397)  
ILAERSAEERRWLKTQGRE (SEQ ID NO: 398)  
LLAERKAEELRWLKTHGRE (SEQ ID NO: 399)  
MLAERKAEERRWLQTHGRE (SEQ ID NO: 400)  
MLAERNAEERRW (SEQ ID NO: 401)  
MFAERKAEESRWLQSQGRE (SEQ ID NO: 402)  
MLEERKAEERRWLKTHGR (SEQ ID NO: 403)  
MLAERKAEERRWLKMQGRE (SEQ ID NO: 404)  
MLAERNAEERRWFYTHGRE (SEQ ID NO: 405)  
MLADGKAEERRWLKTHGLD (SEQ ID NO: 406)  
MIADGKAEERRWLKTHGRD (SEQ ID NO: 407)  
MLADGKAEELRWLKTQGS (SEQ ID NO: 408)  
MLAERNAEERRWLKTHGRD (SEQ ID NO: 409)  
MLADGKAEELRWLKTQGRE (SEQ ID NO: 410)

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## Figure 1-10

ILADGKAEERRWLKTHGRD (SEQ ID NO: 411)  
MLADGMPEERRWLQTHGRD (SEQ ID NO: 412)  
MLADGEAEKRRWLNTHGRD (SEQ ID NO: 413)  
MLADGNAEERRWLMTHGRD (SEQ ID NO: 414)  
MLADGEAEKARWLKTQGRE (SEQ ID NO: 415)  
MLAEGEAEKARWLKTQGRE (SEQ ID NO: 416)  
MLADGKAEERRWLKTQGRE (SEQ ID NO: 417)  
MLAERKAEERRWLSAHVRE (SEQ ID NO: 418)  
LLGERKAEERRWYKTHARE (SEQ ID NO: 419)  
MLAERKAEERRWLMTHGHD (SEQ ID NO: 420)  
MLAERKAEERRWLKSQCLE (SEQ ID NO: 421)  
LLAEREAERERRWFKTHGRE (SEQ ID NO: 422)  
MLADGEAEARRWFNMHGRE (SEQ ID NO: 423)  
MLADGRAEEARWLKTQGSE (SEQ ID NO: 424)  
MLAEGRAEEARWLKTQGSE (SEQ ID NO: 425)  
MLAEREAERARWLKTQGRE (SEQ ID NO: 426)  
MMAERKAEEQRWFDIHGRD (SEQ ID NO: 427)  
LTAERDAEKRRWLLTHGGE (SEQ ID NO: 428)  
MLAERQAEERRWLKSQRGE (SEQ ID NO: 429)  
LLAERKAEERRWFATHGRD (SEQ ID NO: 430)  
MLAEREAELRLWLKSQERA (SEQ ID NO: 431)  
MLAERKAEERRWLKTHGGE (SEQ ID NO: 432)  
CTWTDLESVY (SEQ ID NO: 433)  
HTTNEQFFMC (SEQ ID NO: 434)  
DTWLELESRY (SEQ ID NO: 435)  
HNSSPMVGVT (SEQ ID NO: 436)  
DWQKTIPAYW (SEQ ID NO: 437)  
RWGREGLVAALL (SEQ ID NO: 438)  
WSGTRVWRCVVT (SEQ ID NO: 439)  
MSLLSYLRS (SEQ ID NO: 440)  
LDLLAI (SEQ ID NO: 441)  
RIYGVK (SEQ ID NO: 442)  
MIWHMFMSLLF (SEQ ID NO: 443)  
FFWASWMHLLW (SEQ ID NO: 444)  
FDDCWREREQFLFQAL (SEQ ID NO: 445)  
CGRASECFRLLEM (SEQ ID NO: 446)  
RECFQMLER (SEQ ID NO: 447)  
CSIRWDFVPGYGLC (SEQ ID NO: 448)  
WMQCWDSLSLCYDM (SEQ ID NO: 449)  
ALLMCESKLAECARAR (SEQ ID NO: 450)  
LAHCKKRKEECAAG (SEQ ID NO: 451)  
SIDGVYLRTSRT (SEQ ID NO: 452)  
SIDGVYLRTSRTRY (SEQ ID NO: 453)  
VRWLRGSTLRGLRDR (SEQ ID NO: 454)  
DRGGGTGVVYWWESY (SEQ ID NO: 455)

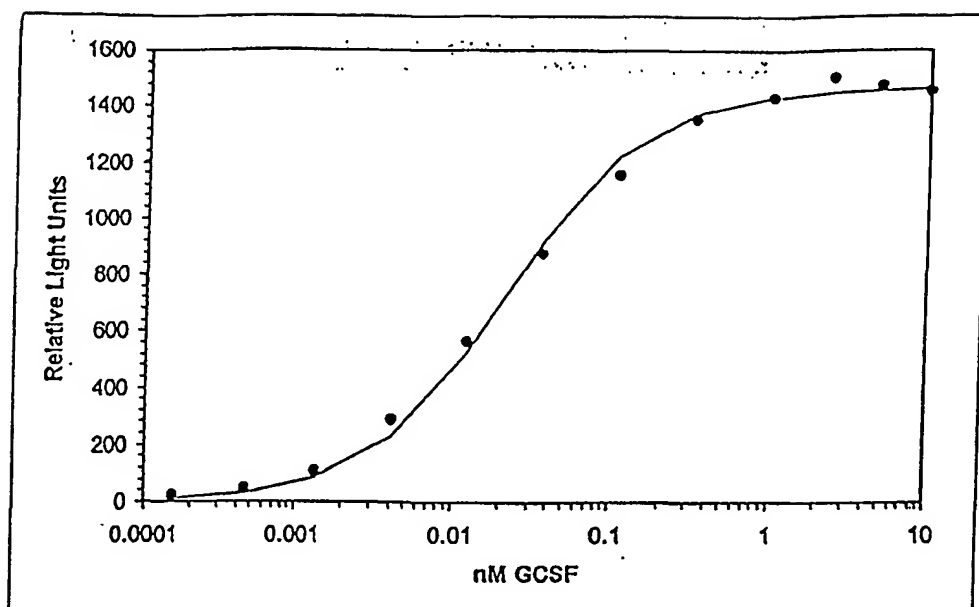
## Figure 1-11

VWGTVGWLEY (SEQ ID NO: 456)  
LMWVSAY (SEQ ID NO: 457)  
RASDEYGALVRFCTNL (SEQ ID NO: 458)  
NYWCDSNWVCEIA (SEQ ID NO: 459)  
LAHCLLRLEECAAG (SEQ ID NO: 460)  
LALCLARLRECAGG (SEQ ID NO: 461)  
CESRLVECSRM (SEQ ID NO: 462)  
LLDIAELKLQECARRCN (SEQ ID NO: 463)  
KLLDIAELKLQECARRCN (SEQ ID NO: 464)  
CSTGGGLTAERDAEKRRWLLTHGGE (SEQ ID NO: 465)  
LTAERDAEKRRWLLTHGGEGG (SEQ ID NO: 466)  
LTAERDAEKRRWLLTHGGEGGK (SEQ ID NO: 467)  
LTAERDAEKRRWLLTHGGEGGGGG (SEQ ID NO: 468)  
LTAERDAEKRRWLLTHGGEGGGGGK (SEQ ID NO: 469)  
ESGWVW (SEQ ID NO: 470)  
NSGWVW (SEQ ID NO: 471)  
SGWVW (SEQ ID NO: 472)  
PLGKCEATCREMARYFN (SEQ ID NO: 473)  
SLQRCEYKLASVRGLCN (SEQ ID NO: 474)  
DLWYLESKLEEAARRCNG (SEQ ID NO: 475)  
PYMGTRSRAKLLRQQ (SEQ ID NO: 476)  
RNAGERRWFKTQGWY (SEQ ID NO: 477)  
MLAERNADDRWFNTHGRD (SEQ ID NO: 478)  
MMADGRLRNSVGLILWCD (SEQ ID NO: 479)  
MLADGRLRNVVG (SEQ ID NO: 480)  
LLADVRRRNGVGLLRMGRD (SEQ ID NO: 481)  
MLADGRLRNFGG (SEQ ID NO: 482)  
TYMTYVYWLC (SEQ ID NO: 483)  
RFGERWGL (SEQ ID NO: 484)  
HWLWWGWNF (SEQ ID NO: 485)  
RECFQMLERC (SEQ ID NO: 486)  
ILAHRNAKERRWFQKHGR (SEQ ID NO: 487)  
CSTGGGLTAERDAEKRRWLLTHGGEK (SEQ ID NO: 489)  
KGGGMLAERKAEERRWFNTHGRE (SEQ ID NO: 490)  
KSTGGGLTAERDAEKRRWLLTHGGE (SEQ ID NO: 491)  
EQSNSGWVWVGGGGCKKKC (SEQ ID NO: 492)



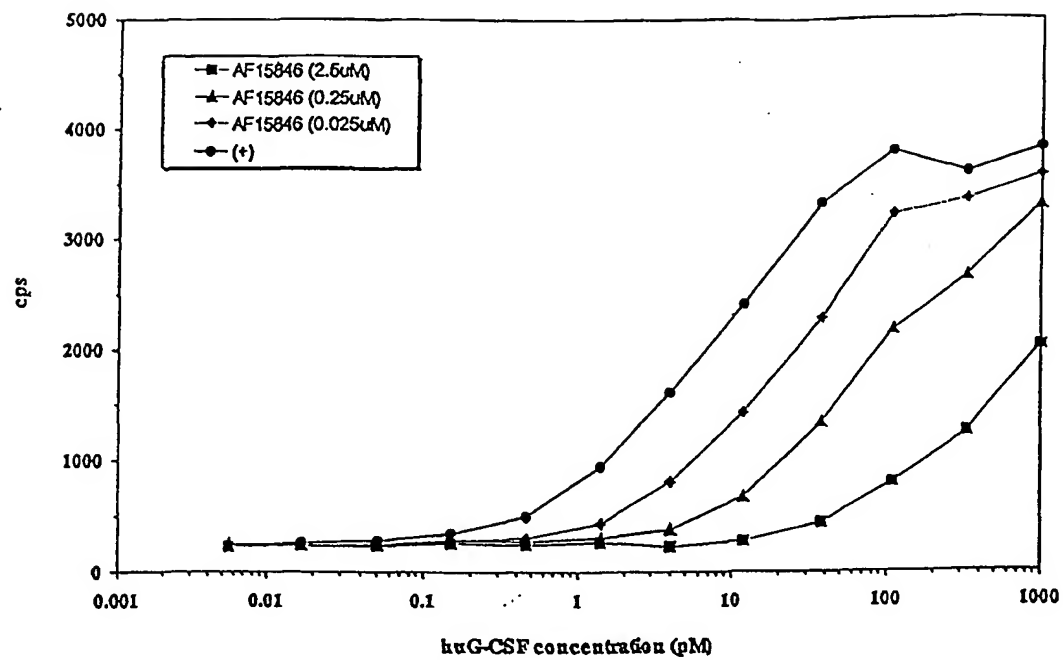
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Figure 2



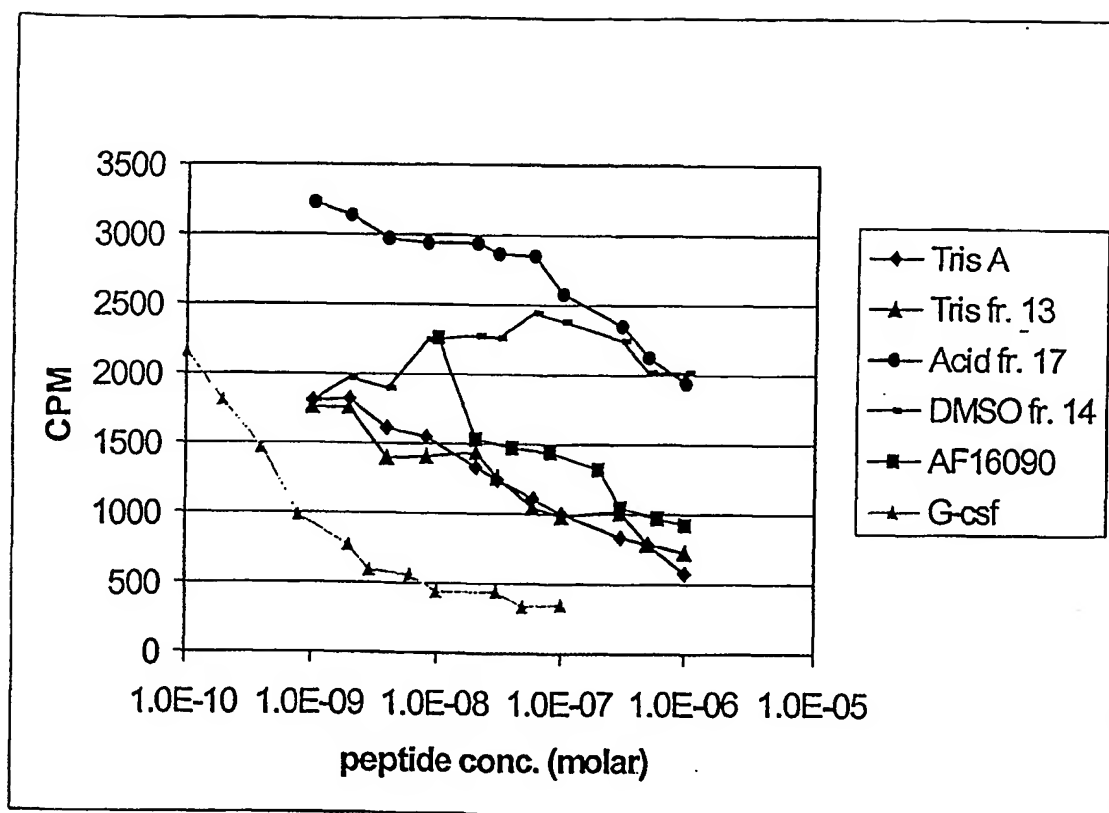
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Figure 3



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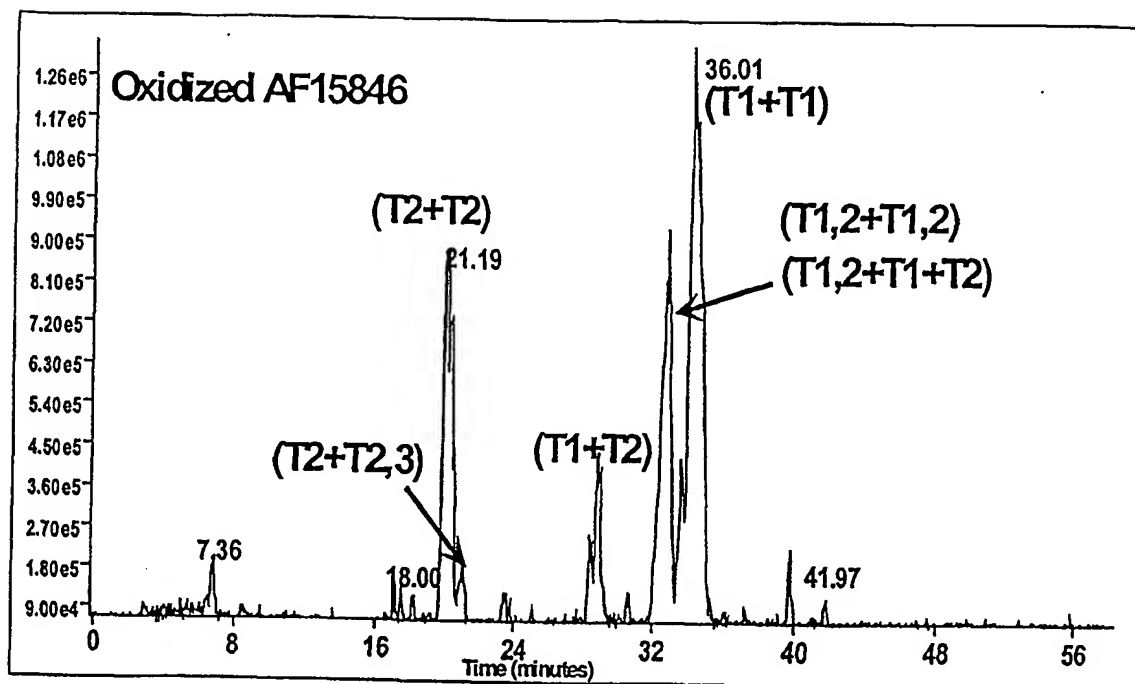
Figure 4



AF16090 = GRTGGGLLDICELKLQECARRCN (SEQ ID NO: 210)

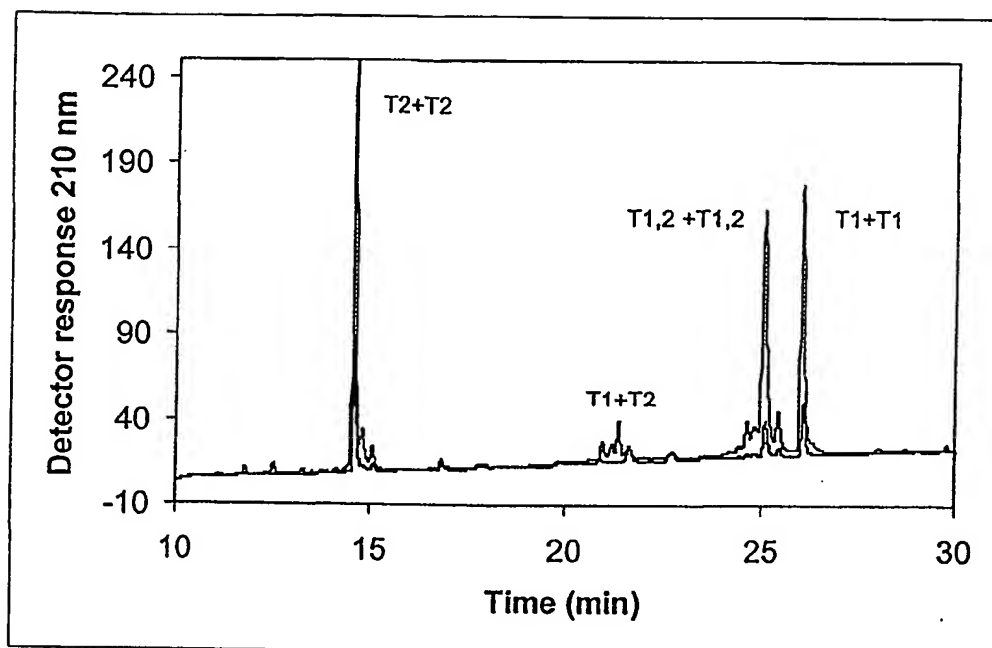
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Figure 5



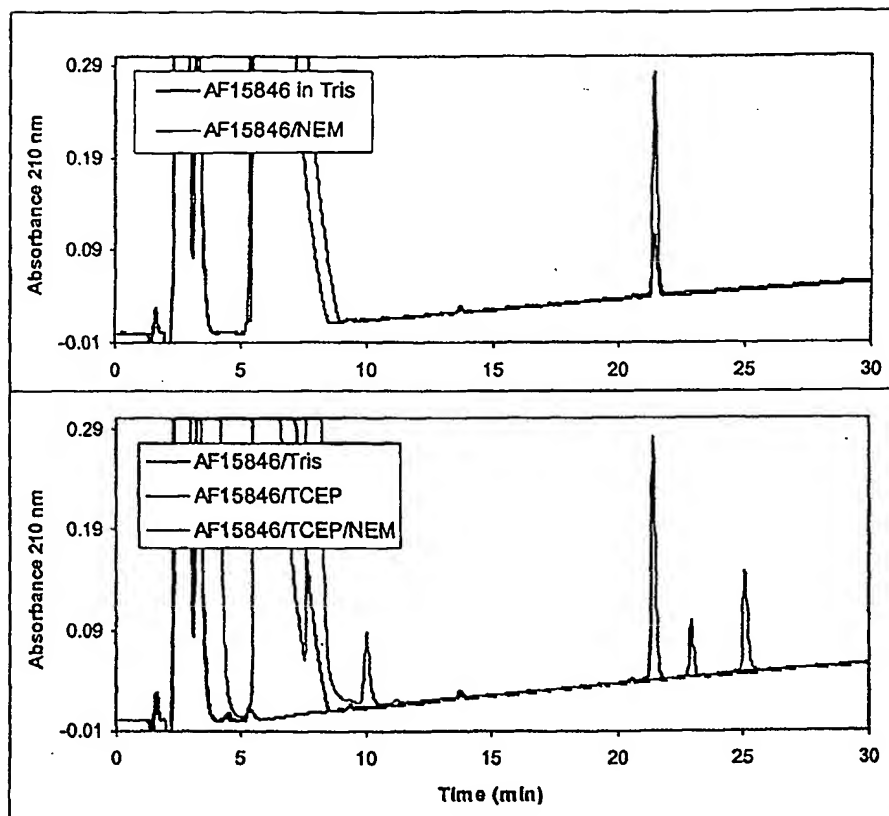
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Figure 6



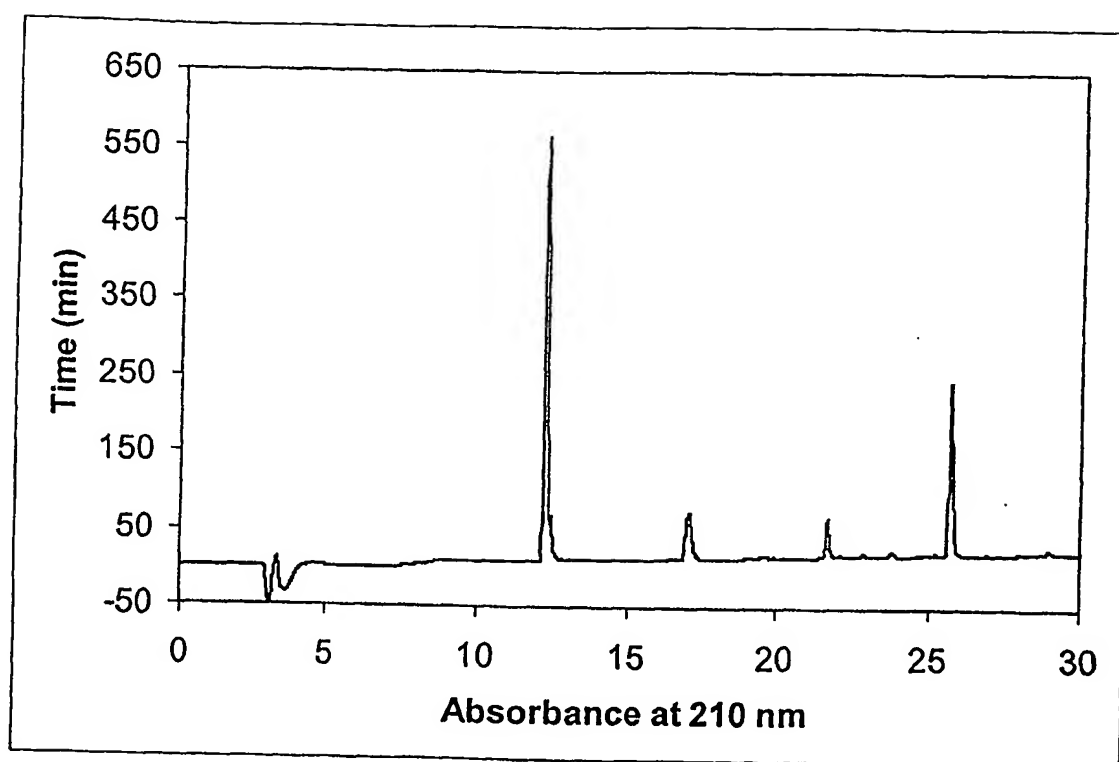
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Figure 7



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Figure 8



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Figure 9A

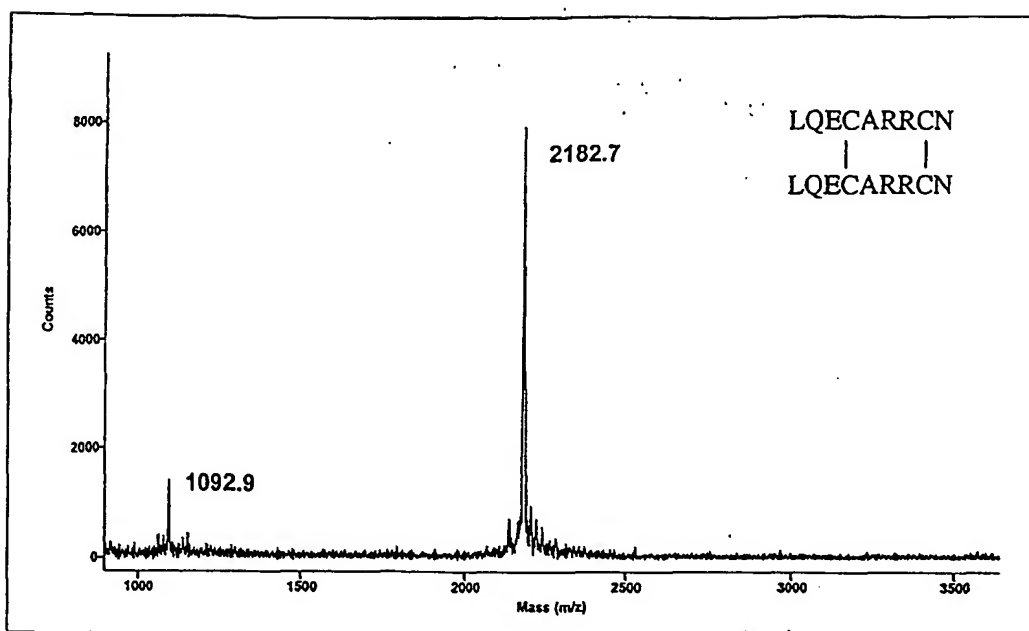
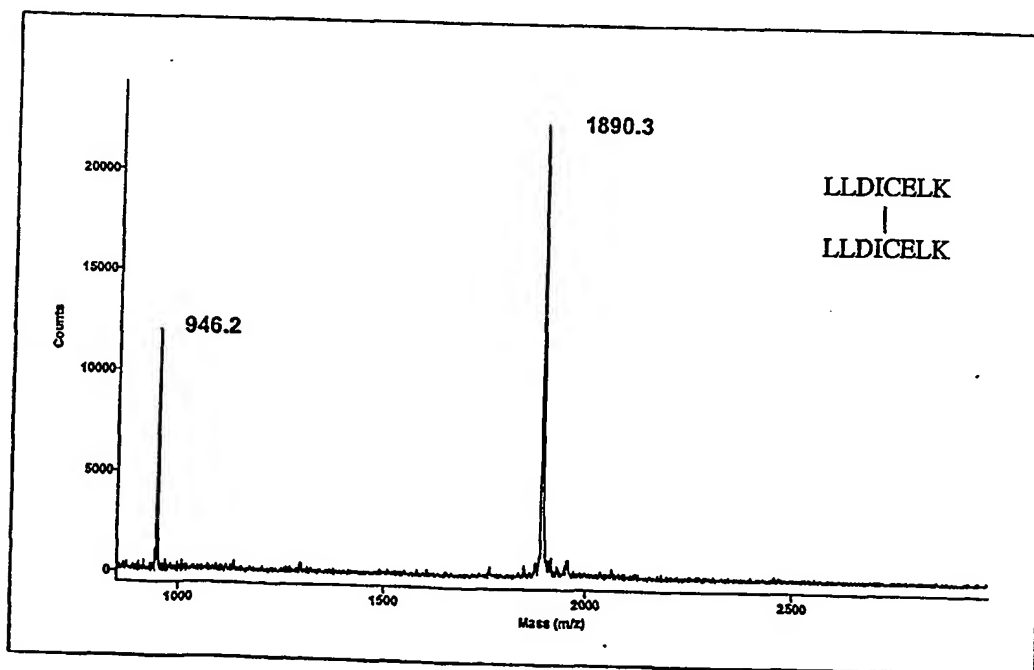


Figure 9B





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Figure 10A

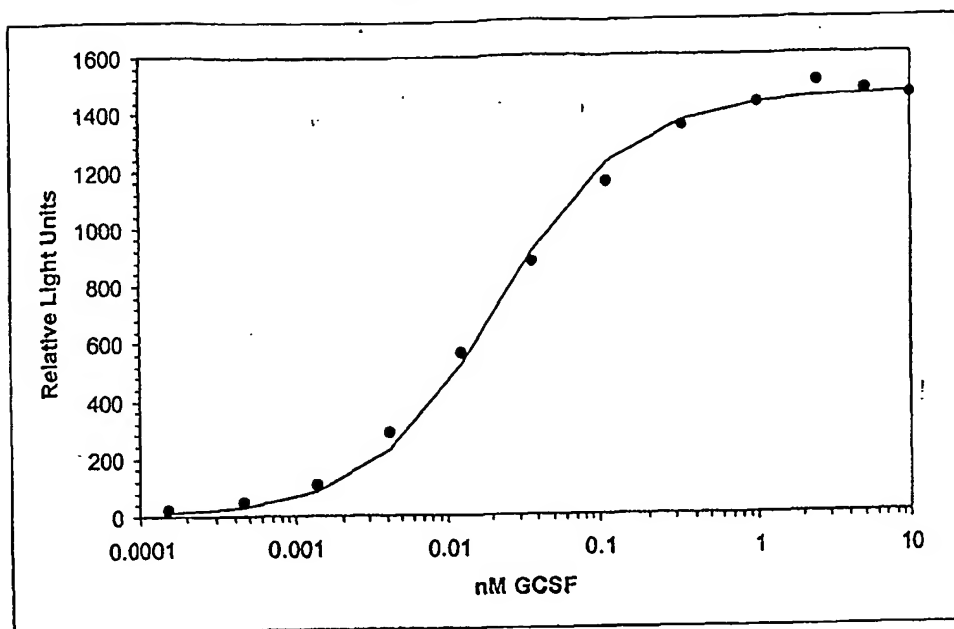
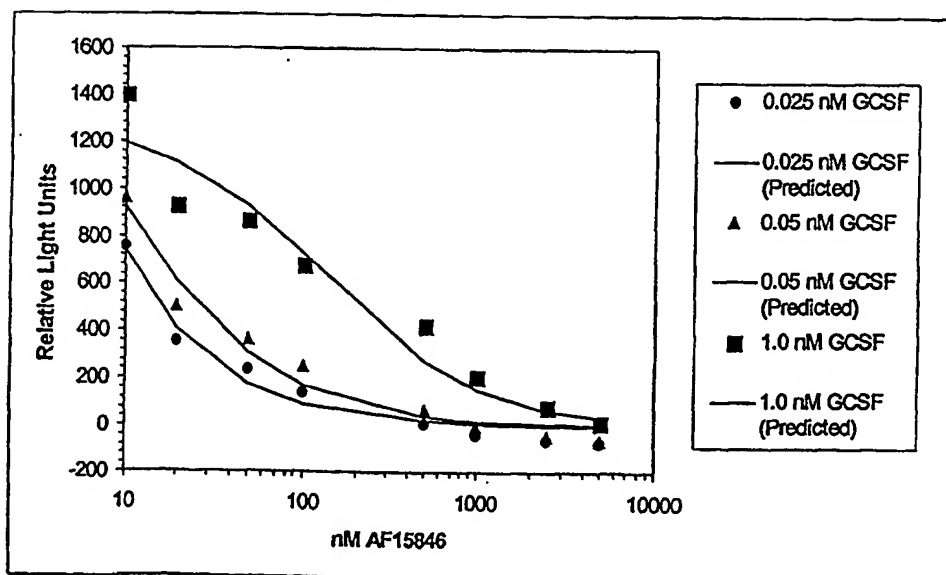


Figure 10B



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Figure 11

